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The Reproductive Ecology of *Astragalus microcymbus*

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THE REPRODUCTIVE ECOLOGY OF *ASTRAGALUS MICROCYMBUS*

by

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ABSTRACT

Astragalus microcymbus Barneby (Fabaceae) is a rare endemic to Gunnison and Saguache counties in western Colorado, ranked as critically imperiled by the Colorado Natural Heritage Program. The purpose of this study was to further investigate this plant's reproductive ecology including reproductive success, pollination ecology and post-primary dispersal seed ecology. To investigate these components of *A. microcymbus* reproduction, this study was guided by the following questions: 1.) What is the reproductive success of *A. microcymbus* as measured by fruit to flower and seed to ovule ratios? Does reproduction vary across sites and what factors influence its reproductive success? 2.) Does *A. microcymbus* require pollinators for successful seed set? If so, what are its primary pollinators? 3.) Does *A. microcymbus* have a viable soil seed bank and how does it vary across the landscape? Can insight into the secondary dispersal mechanisms and occurrence patterns of *A. microcymbus* be gained by the spatial distribution of the seed bank and individuals? Our methods included flower, fruit, seed, and ovule counts, monitoring herbivory interactions, conducting pollinator exclusion experiments, pollinator visitation observations and identification, collecting soil seed bank samples, and describing microhabitat characteristics of individuals. In 2019, we found mean fruit set per plant ranging from 0.25 to 0.30, and seed set per fruit averaging 0.29. In 2020, low reproduction was observed due to the impacts of small mammal herbivory and drought, which limited the reproductive success study and the pollinator exclusion experiment. The genera *Ashmeadiella* (Megachilidae) and *Anthidium* (Megachilidae) were observed visiting and eliciting the pollination mechanism of flowers. *Astragalus microcymbus* exhibited a soil seed bank that decreased in density with increasing distance from parent plant. At the intermediate distance from the parent plant (0.5 m), fewer seeds were found in microsites with higher bare ground exposure, suggesting the process of overland flow or wind influence seed dispersal. Finally, established plants occurred in areas closer to vegetation and with higher vegetation cover, suggesting vegetation patches may serve as seed catches and/or nurse plants facilitating seedling establishment and survival. This information broadens our understanding of the life-history and ecology of this rare endemic and informs conservation efforts.

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INTRODUCTION

Narrow endemism is a condition characterizing species with few local populations restricted in geographical distribution (Kruckeberg and Rabinowitz 1985). This may be caused by the interplay of demographic history (including limited gene flow and population fragmentation) and local adaptation (Jones et al. 2021). Narrow endemics tend to occur in regions with high climatic and geological variation. These landscapes are a major force in the evolution of endemics, facilitating isolation followed by high speciation and niche specialization (Kruckeberg and Rabinowitz 1985, Jones et al. 2021). Their habitats are often unique from the surrounding environment and uninhabitable to widespread species lacking specialized adaptations (Ferreira and Boldrini 2011, Kruckeberg and Rabinowitz 1985, Lavergne et al. 2004). There is still much unknown about endemism, although biologists have suggested that endemic plants remain restricted to their given habitat or niche through competition, poor seed dispersal abilities, reliance on a specific habitat type or interaction, and genetic limitations; or they may simply be a young species that has not yet expanded to its full potential range (Ferreira and Boldrini 2011, Kruckeberg and Rabinowitz 1985, Lavergne et al. 2004). Due to low abundance and narrow range distribution, rare endemic species may be less resilient to habitat loss and the challenges of climate change (Munson and Sher 2015, Neely et al. 2009). Further, small populations, which can be a characteristic of rarity (Rabinowitz 1981), may be more vulnerable to the genetic challenges of inbreeding and genetic drift (Barrett and Kohn 1991). The conservation of rare endemic species and their limited habitat is of critical importance in the effort to preserve the biosphere’s flora, which is being lost to anthropogenic pressures at unprecedented rates (Antonelli et al. 2020).

To implement effective conservation measures, an understanding of the biology and broader ecology around a species reproduction is critical (Falk and Holsinger 1991, Kaye 1999). From the fertilization of an ovule to the germination of a seed, the reproductive cycle of a plant is composed of many different processes, unique to each species as a result of the distinct selective forces that have produced their life-history. Interference with any component of these processes has the potential to contribute to a species' imperilment (Fenner and Thompson 2005, Kaye 1999). An understanding of the life history characteristics of rare endemics may also inform the reasons for its restriction and rarity, as well as what components of its ecology are necessary for its persistence (Jones et. al 2021, Kruckeberg and Rabinowitz 1985, Lavergne et al. 2004).

Astragalus (Fabaceae), the largest genus of flowering plants, contains about 3,000 species (Barneby 1989), a large number of which are recently evolved endemics native to semi-arid climates (Lesica et al. 2006). Western North America harbors 500 of these taxa (Barneby 1964), with about 160 endemics to the Intermountain West (Barneby 1989). According to Jones et al. (2021) divergence of *Astragalus* in this region occurred 130 thousand years ago during the Pleistocene. Large scale climatic changes of this epoch facilitated founder events by creating environmental fragmentation which isolated *Astragalus* populations, prompting local adaptation to distinct habitats (Jones et al. 2021). Many *Astragalus* are adapted to specific climates or soils. Despite having small, isolated populations, rare species in this genus have been found to have high genetic diversity, which may also facilitate the high rates of speciation seen in this genus (Jones et al. 2021). Jones et al. (2021) suggests the seed ecology in *Astragalus* species plays a role in their endemism. *Astragalus* sp. can have long-term soil seed banks containing multiple generations, which may maintain high genetic variation even in small populations. Further, limited seed dispersal may inhibit gene flow and facilitate local adaptation, leading to the diversity of species that occur within this genus (Jones et al. 2021).

Astragalus microcymbus Barneby (Fabaceae), is a rare endemic restricted to the sage-brush steppe in Gunnison and Saguache counties of western Colorado, ranked as critically imperiled (G1/S1) by the Colorado Natural Heritage Program (Neely et al. 2009). This species is a polycarpic perennial that grows in well drained, cobble-clay loam soils on southeast to southwest facing slopes of 9-38 degrees. The annual precipitation in its habitat range is about 25 cm, with average daily temperatures of -20° C (-4° F) to -3°C (26.5°F) in January and 6°C (44°F) to 27°C (81°F) in July (U.S. Fish and Wildlife Service 2009). The average lifespan of an individual is two to four years, but individuals have been observed living up to 17 years (DePrenger-Levin et al. 2013). It emerges in late-May to mid-June and blooms throughout the growing season from late May to mid-October with tiny white and purple papilionaceous flowers (Figure 1). When pollinated, flowers develop into boat shaped pods with green and red patterning (Figure 2), hence the common name skiff milkvetch (U.S. Fish and Wildlife Service 2009).

Figure 1. *Astragalus microcymbus* flowers.

Figure 2. *Astragalus microcymbus* fruit.

Several populations have been monitored since 1995 by the Denver Botanic Gardens (DBG) in a long-term demographic study looking at population trends, life-history events such as dormancy and mast seedings, and the effects of climate and herbivory on population dynamics (DePrenger-Levin et al. 2013, DePrenger-Levin and Hufft 2019). According to this study, yearly variation in reproductive output and life-history expression is correlated with climatic factors. Multiple abiotic and biotic factors must coincide to create conditions that favor *A. microcymbus* reproduction and often these conditions are not met. There is significant year-to-year variation in *A. microcymbus* fruit production, resulting in few masting years. Mast seeding, a reproductive strategy characterized by infrequent years of high reproductive output synchronized amongst individuals in a population, is correlated with high winter precipitation, low spring temperatures, and moderate temperatures during spring and summer in this species (DePrenger-Levin et al. 2013). Individuals may express seasonal dormancy, which may be a life-history strategy to buffer against environmental challenges (DePrenger-Levin and Hufft 2019). High spring and summer temperatures may result in more dormant individuals and fewer reproductive individuals (DePrenger-Levin et al. 2013). *A. microcymbus* undergo fluctuating population sizes of cyclical growth and decline, but overall populations appear stable (DePrenger-Levin et al. 2013).

From 2010-2019 *A. microcymbus* was a candidate for listing under the Endangered Species Act, although due to the current status of studied populations, the decision was made to not provide it federal protections. Despite this listing decision, threats to these populations are present and potentially growing (U.S. Fish and Wildlife Service 2009). Sagebrush steppe is considered one of the most threatened ecosystems in the United States due to habitat fragmentation and degradation (Davies et al. 2011). Further, Colorado is one of the fastest growing states in the US and the anthropogenic impacts inherent in this growth jeopardize the existence of *A.*

microcymbus along with many other plant species (Neely et al. 2009). The town of Gunnison specifically is expected to double in population by 2050 (Colorado Water Conservation Board 2009). Habitat fragmentation from recreation, urban or residential development, the establishment of invasive annual species, small mammal herbivory, and climate change are among the greatest threats to this species (U.S. Fish and Wildlife Service 2009). Climate change poses a significant challenge to this species, as temperatures and drought are expected to increase, with more warmer days in the year and decreased winter snowpack (U.S. Fish and Wildlife Service 2009). There are gaps in our understanding of *A. microcymbus* reproduction, and this limits our ability to conserve its populations and habitat under these pressures.

A deeper understanding of the reproductive biology of *A. microcymbus* will allow us to better understand its life-history, the habitat and communities it relies on, the threats which endanger it, and its potential for persistence. Understudied components of this plant's biology include its breeding system and pollination ecology, seed dispersal mechanisms, and soil seed bank characteristics (DePrenger-Levin et al. 2013, U.S. Fish and Wildlife Service 2009). In addition, quantifying reproductive success, monitoring threats that interfere with reproduction, and describing microhabitat characteristics will contribute to our understanding of *A. microcymbus* reproduction.

Reproductive Success

In angiosperms, reproduction begins with the initiation of flowers, which bear the male (androecium) and female (gynoecium) reproductive structures of the plant. Fertilization is facilitated by pollination, when pollen from an anther is carried to a stigma, grows down the style and into the ovary, and delivers male gametes to female gametes within the ovules. Fertilization initiates the development of an embryo within a seed that derived from the ovule, encompassed by a fruit derived from the ovary, which bear the seeds during development and sometimes into dispersal. The number of flowers that develop into fruits (fruit set or fruit to flower ratio), and the number of ovules that develop into viable seeds (seed set or seed to ovule ratio), represent the pre-dispersal reproductive success of a plant each growing season (Fenner and Thompson 2005). Many factors influence the reproductive success of a plant which can be intrinsic, determined by a plant's biology, or extrinsic, affected by surrounding ecological factors like abiotic growing conditions or biotic interactions (Fenner and Thompson 2005).

Pre-dispersal hazards to the reproductive success of a plant may occur from pollination limitation, ovule abortion, resource limitation, and herbivory or seed predation (Fenner and Thompson 2005, Louda 1982). Pollen limitation is a common occurrence in some plant species and can occur if due to lack of pollen deposition, some of a plant's flowers do not get fertilized and do not develop into fruit or achieve full seed set (Fenner and Thompson 2005). For those ovules that are pollinated, during the process of development fertilized ovules may be aborted if the developing embryo has low fitness due to abiotic stress (Sun et al. 2004), self-fertilization or resource availability (Martin and Lee 1993). Reproduction requires a significant amount of resources, and this can be a limiting factor in the amount of reproduction a plant can successfully complete (Fenner and Thompson 2005). During a plant's reproduction, herbivory and seed predation are interferences that can occur during flower and seed development phases of reproduction (Louda 1982). Flowers and seeds may consist of an important part in the diet of various animals, but the consumption of these structures can be a significant loss to a plant's reproductive success (Fenner and Thompson 2005, Louda 1982).

Flowering plants often produce extra flowers in a given year, some of which do not produce fruit or seed, which may offset these hazards to reproductive success (Fenner and

Thompson 2005). Producing extra flowers may allow plants to take advantage of favorable growing seasons when resources and conditions are ideal for reproduction (Fenner and Thomspon 2005). An additional hypothesis, known as the ovary-reserve hypothesis, suggests that surplus flowers may provide an ovary reserve to the parental plant in case of an unpredictable loss in flowers (Ehrlen 1991).

Plant reproduction can have significant year-to-year variation (Kaye 1999). Masting behavior, when plants reproduce in irregular cycles with infrequent high reproductive years synchronized amongst entire populations or plant communities, is another strategy that may increase a species reproductive success (Fenner and Thompson 2005). Masting may be driven by adaptive benefits, including seed predator satiation and pollination efficiency. Plant populations inundate seed predator pressures by producing a larger than average seed crop so that a larger proportion of seeds are able evade predation and survive until germination (Pearse et al. 2016). Larger floral displays also increase pollinator efficiency, because there is more pollen available across the population (Pearse et al. 2016). At a mechanistic level, masting behavior may be driven by processes including resource constraints or weather cues (Pearse et al. 2016).

Fruit to flower ratios are characteristic of species, and some naturally have low fruit set (Fenner and Thompson 2005). Multiple studies have observed low fruit to flower ratio in rare *Astragalus* (Kaye 1999, Martínez-Sánchez et al. 2011, Searle 2011). Quantifying fruit and seed set in *A. microcymbus* will help to elucidate relationships between flower, fruit, ovule and seed of a plant. Describing the reproductive success of this species is important in determining factors that may interfere with its reproduction, which is necessary in implementing relevant conservation strategies.

Pollination Ecology

An understanding of a plant's breeding system, the resulting system of where male and female gametes come from in fertilization, is fundamental to implementing conservation efforts for a plant species. The primary types of breeding systems are identified as predominantly outcrossing (allogamy), selfing (autogamy or geitonogamy), or mixed mating systems. Because the plant kingdom can have a variety of breeding systems, the genetic challenges of inbreeding may affect plants differently than it does animals (Barret and Kohn 1991). Some plants may have adapted to self-fertilization or may be capable of tolerating certain amounts of self-fertilization (Karron 1991). Species with narrow habitat ranges or narrow distributions may show higher ability for autogamy than widespread species because they have been selected through population bottlenecks (Karron 1989). Similar to animals though, inbreeding can result in the reduction of fitness in offspring. Outbreeding depression can also occur through loss of local adaptation (Barret and Kohn 1991). Both of these forces, and their potential effect on a species' population genetics, need to be considered in the implementation of conservation efforts (Barret and Kohn 1991). Furthermore, understanding the breeding system which a species uses can aid in the interpretation of population genetics and in determining effective population size (Barret and Kohn 1991). The breeding system of *A. microcymbus,* and the degree to which it requires pollinators for successful seed set is unknown. A study of a single individual showed that when caged, this plant did not produce fruit (U.S. Fish and Wildlife Service 2009).

Species that require pollinators for successful seed set may face challenges of pollen limitation as a result of low pollinator abundance or activity, which can occur in fragmented habitats (Nayak and Davidar 2010). Fragmented habitats with increasing isolation may support lower pollinator abundance and diversity (Steffan-Dewenter and Tscharntke 1999). Many populations of *A. microcymbus* are located in habitats fragmented by roads, trails, and grazing (U.S. Fish and Wildlife Service 2009). Further, pollination limitation could be a threat to this species among others, due to the current downward trajectories seen in pollinator abundance worldwide (Potts et al. 2010). If a plant species requires pollinators for successful fruit and seed set, identifying those pollinators is necessary in the conservation of that species because it allows conservation efforts to be extended to these broader communities and the processes which support them.

Papilionaceous flowers of the Fabaceae family are thought to have to evolved for pollination by Hymenoptera (Aronne et al. 2012). The corolla of these flowers is composed of five distinct petals including the banner petal, a pair of wing petals, and a fused pair of keel petals enclosing the gynoecium and androecium. In flowers with this morphology, reproductive structures are only accessible by "tripping" the flower, a mechanism which is triggered when pressure is applied to the keel (Aronne et al. 2012). Bees are thought to be the most efficient insects capable of triggering this mechanism (Aronne et al.2012). Close behavioral observations of the insects visiting papilionaceous flowers are important in differentiating pollinators from non-pollinating visitors (Aronne et al. 2012). In 1989, a black carpenter bee, *Ceratina nanula* and a yellow and brown satyr butterfly, *Coenonympha orchracea ssp. ochracea* were observed visiting *A. microcymbus* (Heil and Porter 1990), although specific behavior was not described. Identification of the pollinators of *A. microcymbus* will inform a significant gap in the current understanding of this species' life-history and ecology.

Post-Primary Dispersal Seed Ecology

Following maturation and primary dispersal from the parent plant, a seed may undergo different pathways including dormancy, germination or senescence (Chambers and MacMahon

1994). The ultimate fate of a seed is determined by both the processes of secondary seed dispersal and seedling survival. Upon primary dispersal from a parent plant, through secondary dispersal a seed may experience subsequent horizontal or vertical movement across the landscape throughout its life until either germination or death (Chambers and MacMahon 1994). During these pathways, seeds are exposed to variable abiotic and biotic interactions that impose selective forces and create a heterogenous pattern of plant occurrence in a population (Schupp 1995). Understanding the post-primary dispersal seed ecology of a species is critical in understanding the processes of a population's regeneration and persistence.

A soil seed bank forms when viable quiescent or dormant seeds become stored in the soil as potential individuals for recruitment in future years (Fenner and Thompson 2005). Seed banks can be either transient, persisting in the soil for less than a year, or short and long term persistent (Fenner and Thompson 2005). Persistent seed banks may contain seeds from multiple generations of genetically unique individuals. These different generations and the genomes they carry contribute to a population's genetic diversity, buffering against genetic challenges like genetic drift (McCue and Holtsford 1998). They provide a pool of genetic diversity, structured by the selection of year-to-year variation of the environmental conditions within which each generation grew (Templeton and Levin 1979). These genetically different individuals can be pulled from the seed bank for recruitment in varying growing conditions of future years, aiding the population during environmental challenges (McCue and Holtsford 1998). Further, as seeds age in the soil, they may accumulate mutations which can increase novel genetic variation and play a role in the patterns of evolution (Levin 1990). The ecological significance of seed banks will increase as climate change is predicted to increase the unpredictability of climatic conditions (Parmesan and Matthews 2005).

The spatial distributions of seed banks are structured not only by dispersal, but also by patterns of germination and senescence. Germination may be facilitated by nurse structures, including rocks and vegetation, which alter microhabitat characteristics (Loayza et al. 2017, Filazzola et al. 2019). In arid and semi-arid environments, environmental variation is extreme and characterized by high temperatures and low precipitation with limited availability of microsites that provide conditions necessary for successful seedling establishment (Bochet 2015, Loayza et al. 2017). These environments tend to experience strong winds and short but intense rainfall events (Breshears et al. 2003, Bochet 2015). Overland flow, slope and wind are important agents in secondary dispersal for desert plants and influence seedling survival as seeds may be relocated to new microsites that better support germination and establishment (Bochet 2015). In arid landscapes, vegetation tends to occur in a patchy distribution with large areas of bare ground, a pattern which is influenced by the processes of overland flow and wind in seed dispersal (Aguiar and Sala 1999). Established vegetation may act as seed sources or seed catches (Bullock and Moy 2004, Caballero et al. 2008), and provide an altered microclimate more suitable for germination (Filazzola et al. 2019, Loayza et al. 2017). These processes are important in patterning seed fate and ultimately population demography.

Water and wind may play a role in seed dispersal of *A. microcymbus* (Heil and Porter 1990, U.S. Fish and Wildlife Service 2009), although this process has not been described. Based on current knowledge, the seed bank of *A. microcymbus* is sparse (DePrenger-Levin et al. 2013, U.S. Fish and Wildlife Service 2009). Considering the influence that a seed bank can have on a species' ability to persist through environmental and genetic challenges, this information is a significant gap in our understanding of this imperiled species. A deeper understanding of the post-primary dispersal seed ecology of *A. microcymbus* will provide further insight into the processes that support its population dynamics.

Study Objectives

This study set out to describe components of *A. microcymbus* life-history including its reproductive success, pollination ecology, and post-primary dispersal seed ecology by addressing the following questions:1.) What is the reproductive success of *A. microcymbus* as measured by fruit to flower and seed to ovule ratios? Does reproduction vary across sites and what factors influence its reproductive success? 2.) Does *A. microcymbus* require pollinators for successful seed set? If so, what are its primary pollinators? 3.) Does *A. microcymbus* have a viable soil seed bank and how does it vary across the landscape? Can insight into the secondary dispersal mechanisms and occurrence patterns of *A. microcymbus* be gained by the distribution of the seed bank and individuals? A greater understanding of these areas of the life-history of *A. microcymbus* will allow us to better understand the characteristics of its ecology which may contribute to its endemism and rarity. Further, moving forward without federal protections, research in the above areas will help agencies and land managers make informed conservation and management decisions that support the persistence of *A. microcymbus*.

METHODS

Study Area

This research was conducted from 2019-2021 in the South Beaver Creek Area of Critical Environmental Concern in Gunnison County near the town of Gunnison, Colorado, USA. This area is managed by the U.S. Bureau of Land Management (BLM) and utilized for recreation (both motorized and non-motorized) and cattle grazing. This study was carried out at five sites within the South Beaver Creek Drainage Area, including Dirty Sock 1 (DS1), Dirty Sock 2 (DS2), South Beaver Creek (SBC), Yucca (YU) and Douglas Fir (DF) (Figures 3 and 4). These sites encompass four geographically distinct occurrences of *A. microcymbus*. Two of the five sites, DS1 and DS2, are near each other and may be one connected population. Average site slope, aspect and elevation ranged from 8.327 – 20.958 degrees, 145.968 – 282.582 degrees, and 2473 – 2499 meters, respectively (Table 2).

Plant communities at studied sites included a shrub canopy of *Artemesia tridentata*, with secondary shrub taxa including *Yucca harrimaniae*, *Purshia tridentata*, and *Crysothamnus depressus*; cacti including *Opuntia polyacantha*; bunch grasses including *Oryzopsis hymenoides*, *Elymus elymoides*, *Hesperostipa comata* and *Poa sp.*; sod-forming grasses including *Bouteloua gracilis*; forbs including *Phlox hoodii*, *Townsendia incana*, *Packera sp.*, *Astragalus anisus* , *Physaria rollinsii, Penstemon teucrioides*; acrocarpous mosses, vagrant lichen, and biological soil crusts. *Juniperus scopulorum* occur on the perimeters of sites.

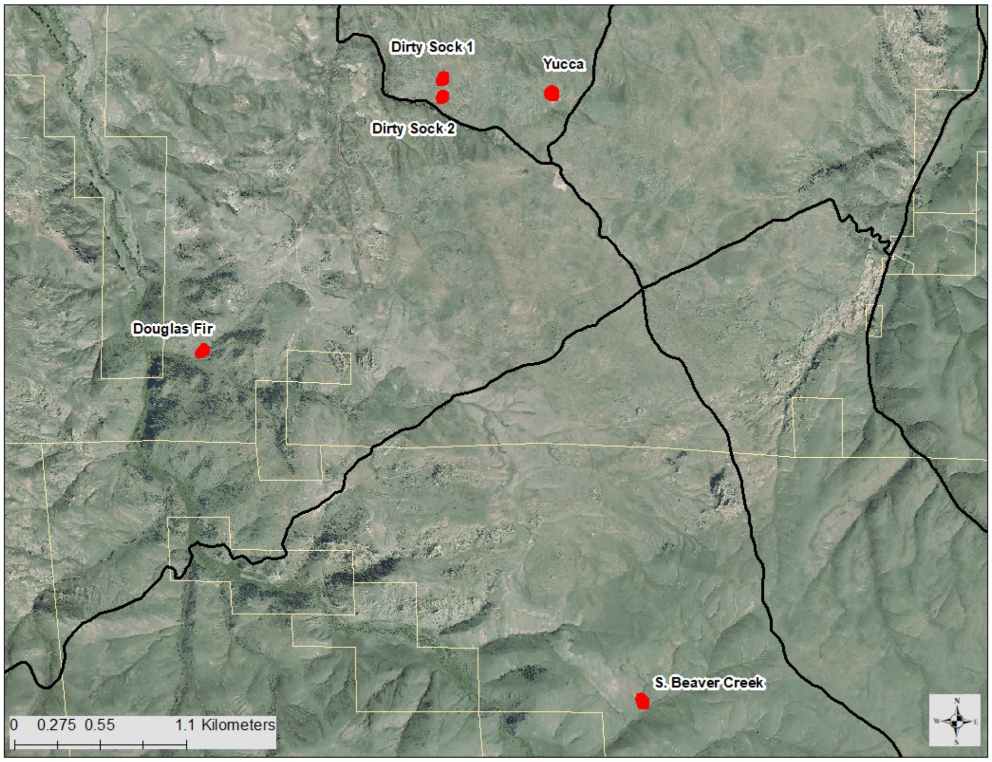

Figure 3. Maps of sites.

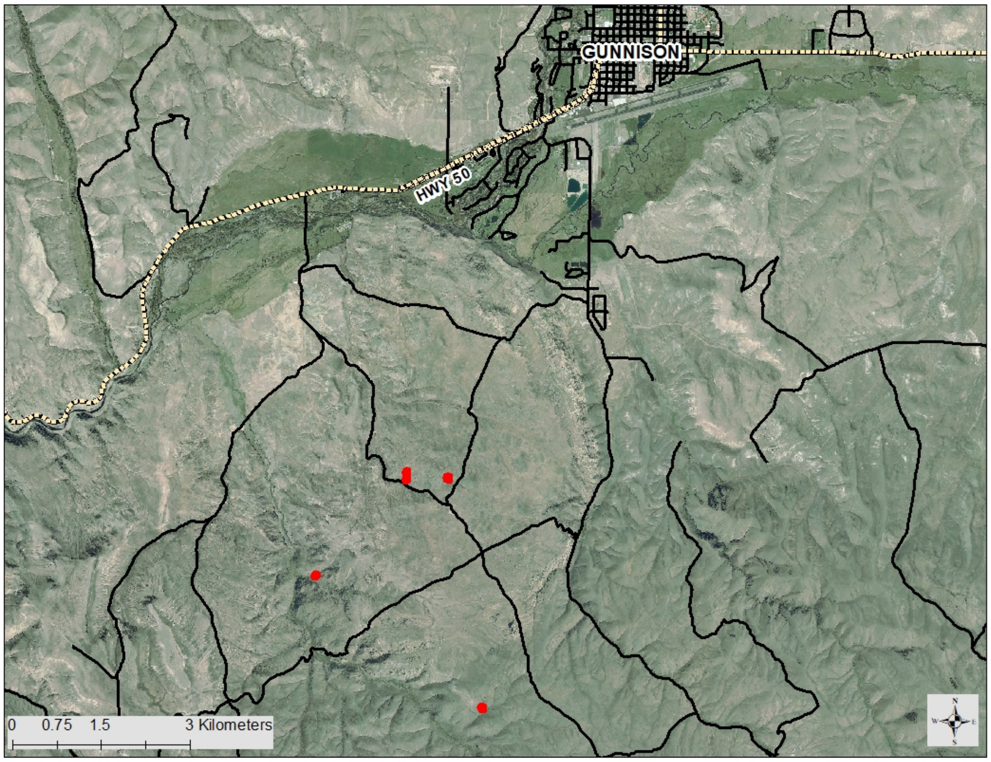

Figure 4. Map of sites and surrounding vicinity.

Population Census and Density

During the process of identifying study individuals for the reproductive success study and pollinator exclusion experiment (see below), population counts and density estimates were made at each site. In 2019, we counted the number of *A. microcymbus* individuals at DS1, DS2, SBC, and YU sites. In 2020, individual counts were only made at DF. The boundary and size of each site was estimated by flagging detected plants. Using nearest neighbor distance measurements, density was estimated at DS1, DS2, SBC, and YU in 2019, and at DS1, DS2, SBC, YU, and DF in 2020. To take these measurements, a transect (50 meters in 2019 and 60 meters in 2020) was placed through the estimated center of the population. The nearest plant at every meter along the transect was identified, and the distance between that focal plant and its nearest neighbor was recorded. In 2020, the methodology used for estimating density differed from 2019. Instead of taking nearest neighbor measurements from the nearest individual to the transect, they were taken from the individuals used in the reproductive success study (see *Reproductive Success,* below). To calculate density, the average distance within a site was calculated and used in the equation:

Equation 1. Equation used to estimate density of *A. microcymbus* plants across sites using nearest neighbor measurements.

$$1 \div (1.67 \times \text{average distance})^2$$

Reproductive Success

2019 Fruit to Flower Ratios-

In order to investigate the reproductive success of *A. microcymbus* as measured by fruit to flower ratios, in the summer of 2019, we randomly selected 50 plants at DS1, DS2 and SBC. These plants were monitored from 1 June through 10 August. The plants used in the reproductive success study were randomly sampled by locating a randomly chosen distance down the transect and a randomly chosen distance to the right or left of the transect, within the estimated boundaries of the site. Distances were chosen using a random number generator. These plants were marked with pin flags and labeled with ID tags attached to the longest stem by cotton thread. Only plants with a max height larger than 10 cm were studied to reduce the chance of sampling individuals not of reproductive age.

The reproductive success of marked plants was quantified at each site over four visits throughout the season. During each visit, we recorded the length of longest stem, number of racemes, number of flowers, number of fruits, mammal herbivory, insect herbivory, and seed predation for each plant. The length of the longest stem was not always able to be tracked throughout the season due to herbivory and tag losses. In these cases, the next longest stem was measured. The number of stems was defined as the number of main stems coming from the base of the plant. The number of racemes included those in bloom, those not yet in bloom and those past bloom. The number of flowers was the total number of flowers per plant at the time of data collection and included open flowers, dried flowers, and pedicel scars where past flowers had grown. The number of fruits was the number of all fruits on the plant at the time data collection was made, including newly initiated fruit to mature fruit beginning to dehisce.

2019 Seed to Ovule Ratios-

In order to investigate the reproductive success of *A. microcymbus* as measured by seed to ovule ratios, during late July to early August 2019, we collected fruit from the reproductive success plants studied at DS1, DS2, and SBC. Three fruits were collected per plant prior to dehiscence. Due to the rarity of this species, collections were only made from plants that had over 10 fruit on the plant at the time of collection. Collected fruit were saved in labelled paper envelopes and stored at room temperature.

In February 2021, fruit were opened, and the number of undeveloped ovules, aborted seeds, young seeds and mature seeds were counted. Categories were based on size, color, fullness, and texture of the ovule or seed. The total ovules count was calculated by adding the number of unfertilized ovules, aborted seeds and developed seeds. The smallest size flecks, visible with a dissecting scope, were categorized as unfertilized ovules. Ovules that had undergone any degree of expansion but were not filled were categorized as aborted seeds. Developed seeds are plump and exhibit a shiny light brown to black seed coat with a subtle pocked texture and were categorized as such based on the presence of these characteristics. Some fruit were collected immature, and seeds were not fully developed. The distinction between young seeds and developed seeds was primarily determined by texture of dried fruit and the color and fullness of the seeds. Young seeds were included in total developed seed counts, assuming they could have completed development given the time, although the viability of young seeds was not tested. Seed rain per plant was estimated by multiplying the maximum number of fruits per plant and the average number of seeds per fruit.

Seed Viability Testing-

In order to determine the seed viability of *A. microcymbus* seeds, in March of 2021, I conducted germination and tetrazolium staining tests on seeds collected for the seed to ovule ratios. All seeds from each site were pooled, and then the pooled seeds were divided into three replicates. According to Seglias (personal communication 2020), during a germination test, *A. microcymbus* seeds required a scarification treatment but not a stratification treatment. For sterilization, seeds were soaked for 1 minute in a 10% bleach mixture, while being agitated, and then rinsed twice in beakers of 200 ml distilled water for 1 minute each (Seglias personal communication 2020). To scarify the seeds, they were initially placed in a vial of sand, and agitated on a vortex mixer for 60 seconds. Seeds were placed in petri dishes on a sheet of filter paper moderately saturated with distilled water. Petri dishes were sealed and placed in a germination chamber set at 20°C with light and 10 °C with darkness, for 12 hours each. Seeds were checked daily and recorded as germinated if at least 1 mm of a radicle emerged from the outer seed coat. This initial scarification treatment did not meet the scarification requirements, because very few seeds were imbibing, so I then scarified these seeds using 220 sandpaper (Seglias personal communication 2020). Seeds were individually sanded until at least one area of the top layer of the seed coat was lightly scratched away. These seeds were then returned to petri dishes with the scarified side facing down, returned to growth chambers, and the checked daily for signs of germination. Seeds were recorded not viable if they rotten or molded. Tetrazolium testing was used on seeds that did not germinate within approximately one week. Tested seeds were bisected with a scalpel and soaked in a 1.0 % tetrazolium solution for 4-6 hours. Seeds that stained evenly bright red were considered viable. Following germination tests, viable seedlings were transplanted into potting soil and transferred to the Denver Botanic Gardens in May of 2021.

2020 Fruit to Flower Ratios-

In order to investigate the reproductive success of *A. microcymbus*, during the summer of 2020, at DS1, DS2, SBC, YU and DF, I monitored 30 plants from 19 June to 15 July. These plants were randomly selected along a 60 meter transect that was placed through the estimated center of the site. To select plants, a random point was located every 2 meters along the transect and at a random distance within 20 meters perpendicular to right or left of the transect. Random distances were generated using a random number generator. The nearest plant to this random point was located and marked for the reproductive study. The distance between that focal plant and its nearest neighbor was measured and used to calculate density. Plants were marked with pin flags and labeled with aluminum ID tags that were staked into the ground with metal anchor pins to avoid the wildlife and cattle interference that caused plants to go missing in 2019. Anchor pins were placed about 6” from the base of the plant. At the same time that reproductive success individuals were identified, the nearest neighbor to these plants at least 1 meter away was also marked and labeled for the pollinator exclusion experiment.

Sites were visited every six days, with all measurements per site taken within three days of each other. At each plant, I marked up to six racemes with thread and counted the number of flowers and number of fruits. In addition, during each visit, I recorded the length of longest stem, number of stems, number of racemes, and mammal herbivory. The number of main stems and racemes were counted using the same method as in 2019. Mammal herbivory was identified by bitten stems. This was distinguished from insect herbivory and damage which appeared restricted to leaves, often leaving behind the rachis and the parts of leaves showing tiny bite marks. Insects damage to *A. microcymbus* may also include girdled stems (DePrenger-Levin and Hufft 2019). If stems were seen on the ground beneath the plant, this was not included as mammal herbivory. Mammal herbivory was accounted for in length of longest stem measurements using a dichotomous variable recording whether the measured stem was grazed or not. In addition, I recorded the degree of plant herbivory based on 4 categories: absent, low, moderate, and high. Absent was no signs of mammal herbivory. Low was if there was some grazing, but it was restricted to the tips of branches. Moderate was if less than half (visually estimated) of the main stems were grazed to 15 cm or less or if less than half of the plants vegetative growth had been grazed. High was if more than half (visually estimated) of the main stems were grazed to 15 cm or more or if more than half of the vegetative growth had been grazed. During the fifth collection period, I recorded the ratio of the number of diagonal stem bites to the number of not diagonal stem bites to quantify herbivory. According to Elbroch (2003), smooth diagonal bite marks are characteristics of lagomorphs and rodent incisors. From 5 August to 17 August three game cameras were set out facing individual plants at SBC and YU to photograph herbivore visitors.

Duration of Flower Bloom and Fruit Maturation-

To inform fruit and flower counts and characteristics of this plant's reproduction, I recorded the blooming and ripening periods for *A. microcymbus*. To make these estimates, during the summer of 2020 I marked 11 flowers and 17 fruit across 4 different racemes on 3 different plants at DS1. These racemes were marked with thread and checked every 24 hours until there were no more flowers and then every two days, until there were no more fruits. Flower age was determined using color. Open flower counts included purple and white to lightly yellow flowers, until they began to dry. Bees were observed visiting lightly yellowed flowers but not dry flowers, and orange anthers were still observed in lightly yellow flowers.

Data Analysis-

In 2019, some plant I.D. tags were lost due to wildlife or cattle disturbance, which reduced our sample size. Plants without observations during at least both of the last two visits were excluded from analysis. The final number of plants that remained at each site was 49 at DS1, 48 at DS2 and 23 at SBC. As reproduction increased throughout the growing season, time taken to complete visits to each site increased and intervals between visits increased, offsetting the visit times across sites, so max counts were used in the analysis.

All analyses were performed in R 4.1.1 (R Core Team 2021). Measures of plant size were transformed with appropriate transformations prior to analyses, as follows. The number of main stems, racemes, flowers and fruits were log transformed. The length of the longest stem was square root transformed. The percent of developed seeds, aborted seeds and undeveloped ovules were arcsine square root transformed. Average total ovules and developed seeds were closest to normal distributions untransformed. Correlations between measures of plant size and reproduction were tested using Spearman's rank correlation coefficient and graphed using the package corrplot (Wei and Simko 2021). Site effect was tested using ANOVA. The length of the longest stem on number of fruit and fruit set were tested using a linear model.

To compare the difference in reproduction between 2019 and 2020, the number of racemes that were measured at each site during the period 30 June through 11 July both years was summarized. The first two weeks of July was estimated to be the height of the flowering season in 2019. The period from 30 June to 11 July was a span closest to this period that each year's collection intervals fell within.

In 2020, due severe impacts of herbivory, only 8 plants at DS1, 5 plants at DS2, 19 at SBC, 9 at YU, and 22 at DF had racemes available to mark. Because there was so little reproduction, fruit to flower ratios were estimated by dividing the total number of flowers recorded across all racemes per site and the total number of fruits recorded across all racemes per site.

Pollination Ecology

Breeding System Study-

In order to determine whether *A. microcymbus* can set fruit in the absence of pollinators, during 15 June to 15 July 2020, at DS1, DS2 and SBC, 1-2 racemes on 30 plants per site were bagged to exclude pollinators. During 6 June to 20 July 2021 at DS1, DF, and Yucca 1-2 racemes were bagged on a total of 44 plants across the three sites. Bags were attached around plants stems and anchored to a pin flag using a twist tie. I monitored bags and counted the total number of flowers and fruits. Results were excluded if bags had holes bitten by rodents or seams were separating.

Insect Visitors-

To identify which pollinating species visit *A. microcymbus*, during the period 22 June to 3 July I made behavior observations of insect visitors that tripped the keel of *A. microcymbus* flowers, eliciting the pollination mechanism. Collections were made of insects that tripped the keel as well as those that did not. Due to low *A. microcymbus* reproduction and limited availability of reproducing plants in 2020, insect collections were only made at SBC. Also due to low reproduction, visitation rates were not collected. Captured insects were killed either by using ethyl acetate or were kept on ice and brought back to the lab and frozen. These insects were pinned, identified to genus and are deposited in the Western Colorado University Faunal Museum in Gunnison, CO and the USDA ARS U.S. National Pollinating Insects Collection in Logan, UT.

Post-Primary Dispersal Seed Ecology

Soil Seed Bank Characteristics-

In order to determine whether *A. microcymbus* has a soil seed bank, I took samples at 0.0 meters, 0.5 meters, and 1.0 meter downhill from 10 focal plants per site. Preliminary soil samples were taken at YU 9 November 2019 and 11 June 2020. At the time June 2020 samples were taken, there was no signs that plants in the area of samples had begun fruit production that year, suggesting any seeds present in the soil were from 2019 or earlier. In November 2020, soil samples were taken at DS1, DS2, SBC, DF, and YU. Plants were sampled every meter, along a 10 meter transect at a random distance to the right or left, within 10 meters on either side. Only plants that had signs of growth from 2019 or had at least 4 stems were sampled to exclude non-reproductive individuals. During November 2020, only individuals that did not have an individual growing within a 1-meter radius were sampled to reduce the potential of observing seeds from individuals other than the focal plant. This sampling criteria of excluding nearby individuals was not included during the November 2019 and June 2020 samples, which inhibited our ability to statistically analyze the difference in seed bank density over time (November 2019 to November 2020) from these three collection periods of YU samples.

In order to inform seed bank spatial distribution and seed dispersal ecology, during November 2020, microhabitat characteristics within a 6 cm radius of the center of the location that soil samples were taken were described using the same methodology as the cover estimates in the plant microhabitat characteristics study (see "*Microhabitat Characteristics- Cover Estimates*").

To collect a soil sample, I pressed a cylindrical soil core (6 cm in diameter by 3.5 cm in height) into the soil until it was flush with the surface (3.5 cm deep), and using a trowel, lifted the contents into a paper bag. This meter-long distance began slightly offset from the base of the plant to reduce disturbance to roots. Organic matter present on the soil surface was included in soil collection. If the distance (0.0 m, 0.5 m, or 1.0 m) fell on a rock, I took the sample on the closest uphill edge of the rock. If the rock's longest length was less than 10 cm, I moved the rock and soil beneath it was taken.

Contents of the soil were brought back to the lab and separated using 16 meshes per inch soil sieves. The number of seeds per sample was recorded and these were stored by sample in labeled paper envelopes. All seeds were pooled by site and separated into three replicates. Seed viability was tested following the same methods used in the reproductive success study. All viable seedlings were transplanted into potting soil and transferred to the Denver Botanic Gardens in May 2021.

Data Analysis-

Microhabitat types were aggregated into coarser categories including bare soil, rocks (cobbles and boulders), and vegetation (soil crusts, vagrant lichen, moss, forbs, bunch grasses, sod-forming grasses, prickly pear cactus, yucca, live big sagebrush, dead big sagebrush, secondary shrub canopies, twigs and branches). The effect of distance from focal plant and percent cover of each microhabitat type was analyzed with R version 4.1.1 (R Core Team 2021) using a Bayesian linear mixed effects model from the package rstanarm version 2.21.1 (Goodrich and

Brilleman 2020). This model has a default log link function that linked the predictors (distance from plant and microhabitat cover) to the response variable (number of seeds). This model used a negative binomial distribution, and the default weakly non-informative prior in rstanarm. The seed was set prior to running the model to create replicability and ran the model with 8,000 iterations. Because this model is using a nonlinear log link function, values of percent cover that were within the 90th quantile for each microhabitat type were used to graph the model.

This model included random effects representing correlated group-level intercepts, R_G, where $_G$ is the combination of plant ID, nested within site. The random effects were assumed to be normally distributed with a mean of 0 and a standard deviation of phi: $R_G \sim N(0, \Phi)$. The probability distribution of the response variable was assumed to be negative binomial with mean α and a reciprocal dispersion δ: $y \sim NB2(\alpha, \delta)$. In the following equations, β_0 is the intercept, β_{1-8} are regression coefficients, B is the proportional exposure of bare ground, R is the proportional cover of rocks, and V is the proportional cover of vegetation.

Equation 2. The model of the effect of distance from focal plant on the seed count.

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2$$

Equation 3. The model of the effect of the interaction between distance from focal plant and percent exposure of bare ground on the seed count.

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2 + B * \beta_3 + D_{d=0.5} * B * \beta_4 + D_{d=1} * B * \beta_5$$

Equation 4. The model of the effect of the interaction between distance from focal plant and percent cover of rocks on the seed count.

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2 + R * \beta_3 + D_{d=0.5} * R * \beta_4 + D_{d=1} * R * \beta_5$$

Equation 5. The model of the effect of the interaction between distance from focal plant and percent cover of vegetation on the seed count.

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2 + V * \beta_3 + D_{d=0.5} * V * \beta_4 + D_{d=1} * V * \beta_5$$

Equation 6. The model of the effect of the interaction between distance from focal plant, percent exposure of bare ground, and rock cover on the seed count:

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2 + B * \beta_3 + R * \beta_4 + D_{d=0.5} * B * \beta_5 + D_{d=1} * B * \beta_6 \\ + D_{d=0.5} * R * \beta_7 + D_{d=1} * R * \beta_8$$

Equation 7. The model of the effect of the interaction between distance from focal plant, percent exposure of bare ground, and vegetation cover on the seed count:

$$\log(\propto) = \beta_0 + D_{d=0.5} * \beta_1 + D_{d=1} * \beta_2 + B * \beta_3 + V * \beta_4 + D_{d=0.5} * B * \beta_5 + D_{d=1} * B * \beta_6 \\ + D_{d=0.5} * V * \beta_7 + D_{d=1} * V * \beta_8$$

Microhabitat Characteristics of Established Plants-

In order to inform occurrence patterns of *A. microcymbus*, I took distance measurements and estimated percent cover around the microhabitat of established individuals. I collected data during the first two weeks of October in 2020 and from late July to mid-August 2021. Due to challenging growing conditions in 2020 including drought and high levels of herbivory, many plants had little to no vegetative growth by mid-summer. However, following a large precipitation event in September 2020, many plants produced above ground growth by October.

During both years, at each site a transect was laid at the estimated the center of the population, and a random point was located every two meters at a random distance between 1 and 10 meters to the right or the left of the transect. Random numbers were generated using a random number generator. From this random point, I located the nearest *A. microcymbus* individual. Between the two years, sampling criteria for choosing individuals changed. In 2020, due to limited availability of plants, I sampled any *A. microcymbus* plants including those without live growth that still had stems from 2019's growing season. In 2021, I only sampled plants that had vegetative growth.

Distance Measurements-

Distances to the nearest rock and vegetation patch were taken at DS1, DS2, SBC, and DF in 2020, and in 2021 at DS1, DS2, SBC, and YU. I measured distance to the nearest rock above 40 mm at its longest length and the nearest vegetation patch above 40 mm at its longest length. For rocks, distance was measured to the edge of the rock where it met the ground. For the vegetation patch, distance was measured to the beginning of its visually estimated zone of canopy cover. Live and dead sagebrush were included in vegetation patch measurements. Sagebrush leaf litter was included reasoning that leaf litter was both the product of the shrub's canopy cover and played a role in and was reflective of the shrub's influence.

Distances to the nearest sagebrush were taken at all five sites during 2020. At random points and the center of *A. microcymbus* plants, I measured the distance to the nearest live and dead *Artemesia tridentata* individual. Distance measurements only included *A. tridentata* individuals with a canopy above 24 cm in diameter. Measurements were made from the center of the random point or the center of the *A. microcymbus* plant to the main stem of the sagebrush. *Artemesia tridentata* individuals were recorded as dead if they had less than 8% leaves (estimated visually) and had a canopy diameter of at least 24 cm, or if there was no remaining canopy, but had a main stem length of at least 24 cm.

Microhabitat Cover Estimates-

In 2020 at all five sites and in 2021 at DS1, DS2, SBC, and YU, I described microhabitat structure by estimating foliar and rock cover within a 6 cm radius (12 cm diameter plots) of the center of random points and *A. microcymbus* plants. Rock size was categorized based on the Wentworth scale for classifying sediments (Wentworth 1922). Rocks were measured at their longest length. Any sediments smaller than cobbles were categorized as bare soil. Percent total area of cobbles and boulders was recorded. Vegetation was also recorded by species, or larger categories for grasses, mosses, lichen and soil crusts. Area cover of fallen twigs, branches, and dead mainstems was also estimated. *Artemesia tridentata* individuals were recorded as dead if they had less than 8% leaves. Leaf litter and senesced plant growth still attached to the base of shrubs, grasses and forbs were recorded with the total estimate of that individual's live growth. Finally, area cover of mosses, lichen and biological soil crusts was estimated. If a category was not present, it was recorded as zero. If there were layers of vegetation in a plot, the total percent exceeded 100%. Cover area of focal *A. microcymbus* plants were ignored in estimates.

Data Analysis-

Distance Measurements- Distance to nearest rock, vegetation patch, live *A. tridentata* and dead *A. tridentata* were compared between the focal groups, random point and *A. tridentata,* using a Kruskal-Wallis test in R version 4.1.1 (R Core Team 2021). Live *A. tridentata* and dead *A. tridentata* were combined to make a third category of all *A. tridentata*. They were combined by picking the lowest distance of each two categories, which would have been the closest *A. tridentata* in either category.

Microhabitat Cover Estimates- Observed microhabitat types were clumped by structural type into coarser categories including bare soil, rocks (cobbles and boulders), and vegetation (soil crusts, vagrant lichen, moss, forbs, bunch grasses, sod-forming grasses, prickly pear cactus, yucca, live *A. tridentata*, dead *A. tridentata*, secondary shrub canopies, twigs and branches). Microhabitat percent cover was analyzed through ANOVA in R version 4.1.1 (R Core Team 2021) using a 3-way interaction between type of point measured (*A. microcymbus* or random), microhabitat type and site as the independent variable and microhabitat percent cover as the dependent variable. Pairwise interactions between type of point and microhabitat type and type of point, microhabitat and site were analyzed using emmeans (Lenth 2021).

RESULTS

Population Census and Density

In 2019, the number of individuals counted was 315 at DS1, 736 at DS2, 559 at SBC, and 1177 at YU. In 2020, there were 83 individuals counted at DF. Based on density calculations, in 2019, the number of plants per m^2 was 0.3 at DS1, 0.74 at DS2, 0.95 at SBC, and 0.76 at YU. In 2020, the number of plants per m^2 was 0.4 at DS1, 0.3 at DS2, 0.55 at SBC, 0.28 at YU and 0.33 at DF (Table 1). Average site slope, aspect and elevation ranged are shown in Table 2.

Table 1. The number of individuals recorded during site census and the density calculated from nearest neighbor distance measurements.

Site	Number of individuals	Year number of individuals were counted	2019 Density (plants per m^2)	2020 Density (plants per m^2)
DS1	315	2019	0.3	0.4
DS2	736	2019	0.74	0.3
SBC	559	2019	0.95	0.55
YU	1177	2019	0.76	0.28
DF	83	2020	no data	0.33

Table 2. The average slope, aspect and elevation for each site.

Site	Slope (degrees)	Aspect (degrees)	Elevation (m)
DS1	14.13	282.58	2473
DS2	8.33	226.41	2457
SBC	12.37	145.97	2499
DF	20.96	164.49	no data
YU	14.32	181.76	no data

Reproductive Success

There was marked difference in reproduction between 2019 and 2020. During 30 June – 11 July, the average number of racemes across sites was 47.25 (SD = 60.82, SE = 5.50, *n* = 122) in 2019 and 1.83 (SD = 5.61, SE = 0.46, *n* = 150) in 2020 (Figure 5). The high count for racemes during this period was 361 in 2019 and only 37 in 2020. In 2019, the number of racemes was strongly correlated with both the number of flowers (r = 0.97, $P < 0.001$) and fruit (r = 0.95, $P < 0.001$) (Figure 8), suggesting the number of racemes is a good measure of the amount of reproduction produced by a plant.

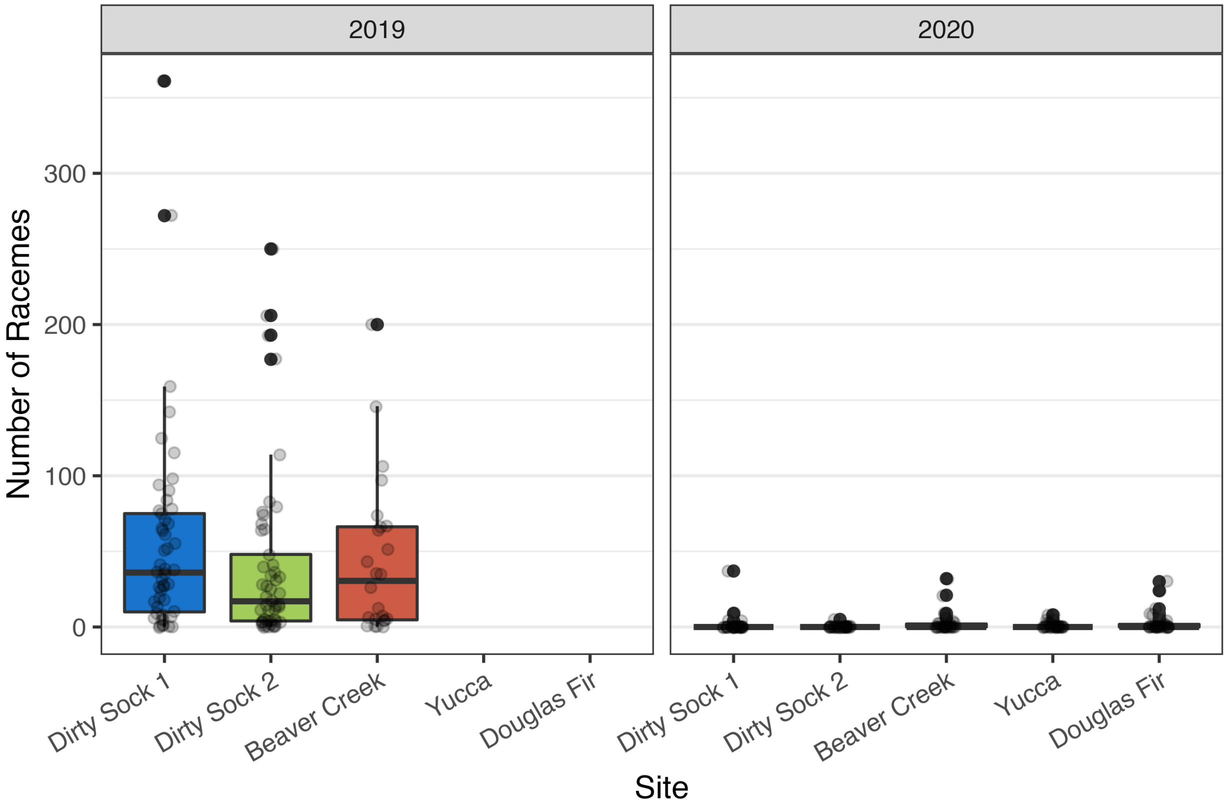

Figure 5. The number of racemes counted per plant at each site during 30 June to 11 July in 2019 and 2020. Number of racemes represents the amount of reproduction that occurred between these years.

2019 Fruit Set-

In 2019, the average max number of flowers counted per plant was 1014 (SD = 1298, SE = 185, *n* = 49) at DS1, 930 (SD = 1408, SE = 199, *n* = 50) at DS2, and 571 (SD = 756, SE = 154, *n* = 24) at SBC. The average max number of fruits counted per plant was 220 (SD = 278, SE = 39.8, *n* = 49) at DS1, 207 (SD = 322, SE = 45.5, *n* = 50) at DS2, and 189 (SD = 296, SE =

60.4, *n* = 24) at SBC. The average fruit to flower ratio calculated from the max flowers and max fruit was 0.22 (SD = 0.14, SE = 0.0197, *n* = 24) at DS1, 0.25 (SD = 0.156, SE =0.0223, *n* = 49) at DS2, and 0.28 (SD = 0.10, SE = 0.02, *n* = 24) at SBC (Figure 6), averaging 0.25 across sites. The average fruit to flower ratio calculated from counts taken 30 June to 11 July was 0.31 (SD = 0.19, SE =0.03, *n* = 46) at DS1, 0.27 (SD = 0.16, SE = 0.02, *n* = 45) at DS2, 0.32 at SBC (SD = 0.13, SE = 0.03, *n* = 21), averaging 0.30 across all sites.

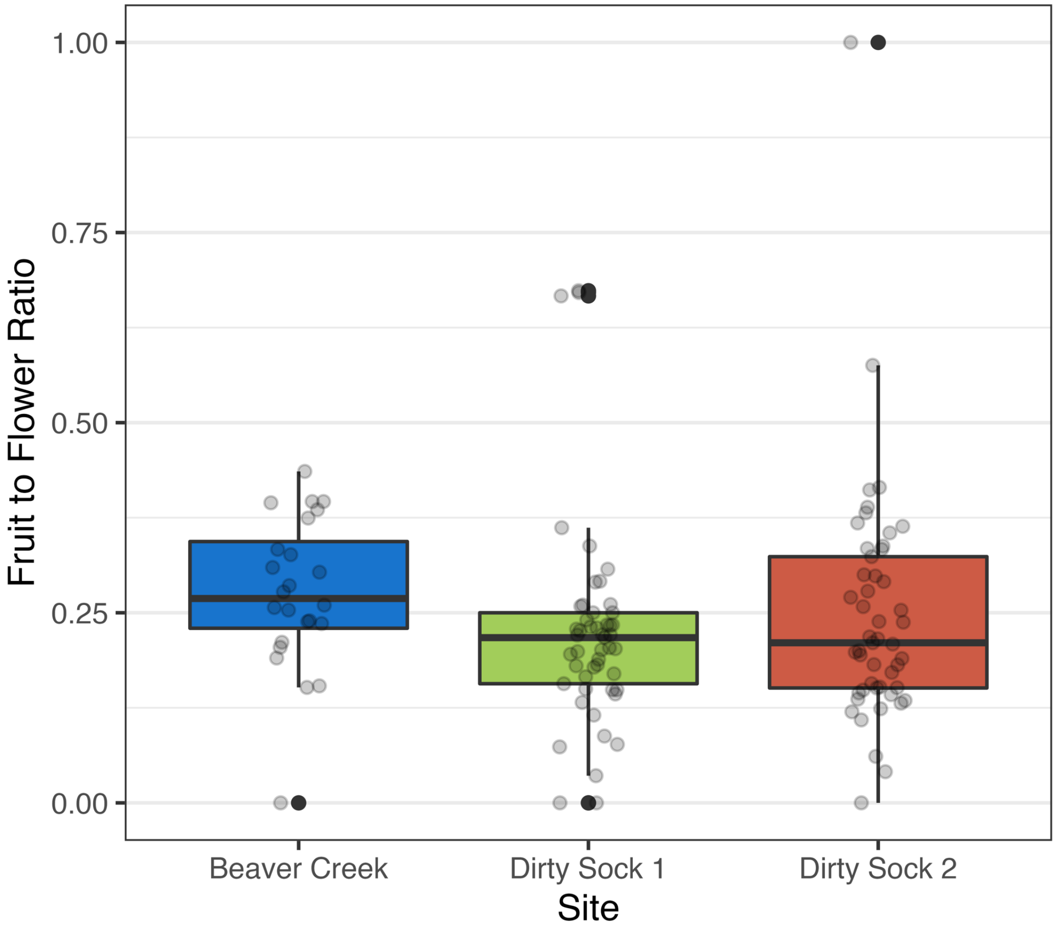

Figure 6. The fruit to flower ratios calculated per site in 2019.

2019 Seed Set-

Fruits were collected from 40 plants at DS1, 28 at DS2, and 22 at SBC. At all sites combined, the average number of total ovules counted within collected fruit was 4.1 (SD = 1.11, SE = 0.12, n = 90). The maximum number of total ovules observed within collected fruit was 6. The average number of seeds per fruit at all sites combined was 1.17 (SD = 0.71, SE = 0.07, n = 90). The average seed to ovule ratio per fruit at all sites combined was 0.29 (SD = 0.18, SE = 0.02, n = 90). The average number of aborted seeds per fruit at all sites combined was 1.39 (SD = 0.99, SE = 0.11, n = 90). The average aborted seed to ovule ratio per fruit at all sites combined was 0.33 (SD = 0.22, SE = 0.02, n = 90). The average number of undeveloped ovules per fruit at all sites combined was 1.53 (SD = 0.93, SE = 0.10, n = 90) (Table 3). The average undeveloped ovule to ovule ratio at all sites combined was 0.37 (SD = 0.22, SE = 0.03, n = 90). (Table 3 and 4, Figure 7).

Seed rain per plant was estimated by multiplying the average maximum fruit per plant and the average number of seeds per fruit. The estimated seed rain per plant was 247 at DS1 (220 fruit per plant × 1.12 seeds per fruit), 240 at DS2 (207 fruit per plant × 1.16 seeds per fruit), and 240 at SBC (189 fruit per plant × 1.27 seeds per fruit).

Table 3. Average number of total ovules, developed seeds, aborted seeds, unfertilized ovules, per fruit at each site. At DS1, *n*=40, at DS2, *n*=28, at SBC, *n*=22.

Site	Total ovules	Total developed seeds	Total aborted seeds	Total undeveloped ovules
DS1	4.15 (SD = 1.13, SE = 0.18)	1.12 (SD = 0.65, SE = 0.10)	1.12 (SD = 0.90, SE = 0.14)	1.92 (SD = 0.88, SE = 0.14)
DS2	4.18 (SD = 0.99, SE = 0.187)	1.16 (SD = 0.77, SE = 0.15)	1.72 (SD = 1.15, SE = 0.22)	1.3 (SD = 0.94, SE = 0.18)
DS3	3.95 (SD = 1.23, SE = 0.26)	1.27 (SD = 0.75, SE = 0.16)	1.43 (SD = 0.81, SE = 0.172)	1.11 (SD = 0.73, SE = 0.16)

Table 4. Average percentage of developed seeds, aborted seeds and undeveloped ovules to average total ovules per fruit at each site. At DS1, *n*=40, at DS2, *n*=28, at SBC, *n*=22.

Site	% developed seeds	% aborted seeds	% undeveloped ovules
DS1	28% (SD = 0.17, SE = 0.03)	27% (SD = 0.20, SE = 0.03)	46% (SD = 0.19, SE = 0.03)
DS2	27% (SD = 0.17, SE = 0.03)	41% (SD = 0.27, SE = 0.05)	32% (SD = 0.24, SE = 0.04)
SBC	34% (SD = 0.20, SE = 0.04)	36% (SD = 0.15, SE = 0.03)	29% (SD = 0.18, SE = 0.04)

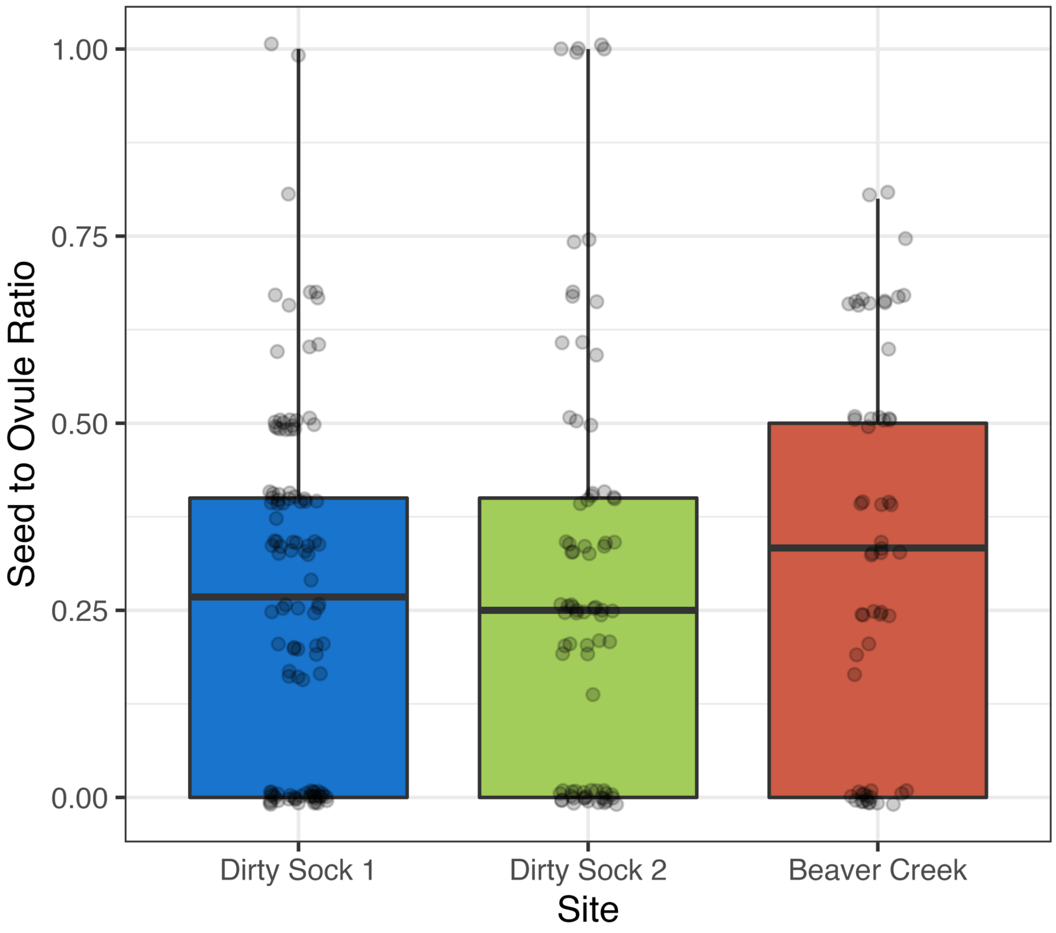

Figure 7. The seed to ovule ratios calculated per site in 2019.

Seed Viability-

The average percent of viable seeds across all three sites was 89%. The average percent of viable seeds across the three replicates was 97% at DS1, 93% at DS2, and 78% at SBC.

Factors Influencing Reproduction-

Across the measures of reproduction collected, highest correlations were between flowers and fruit (r = 0.97, P <0.001), racemes and flowers (r = 0.98, $P < 0.001$), and racemes and fruit (r = 0.95, $P < 0.001$). Measures of plant size, length of longest stem and number of main stems, were weakly correlated (r = 0.46, $P < 0.001$). Length of longest stem was correlated with racemes (r = 0.73, *P* < 0.001), absolute count of flowers (r = 0.75, *P* < 0.001), and absolute count of fruits (r = 0.74, *P* < 0.001). Length of longest stem was not correlated with fruit set (maximum fruit counts divided by maximum flower counts) (r = 0.05, *P* = 0.59) or seed set (r = 0.06, *P* = 0.63). Absolute count of fruit was weakly correlated with fruit set (r = 0.22, *P* = 0.05) but not seed set (r = -0.04, *P* = 0.72) (Figure 8). Absolute count of flowers was not correlated with fruit set (r= -0.8, *P*= 0.50), or seed set (r = 0.03, *P*=0.77).

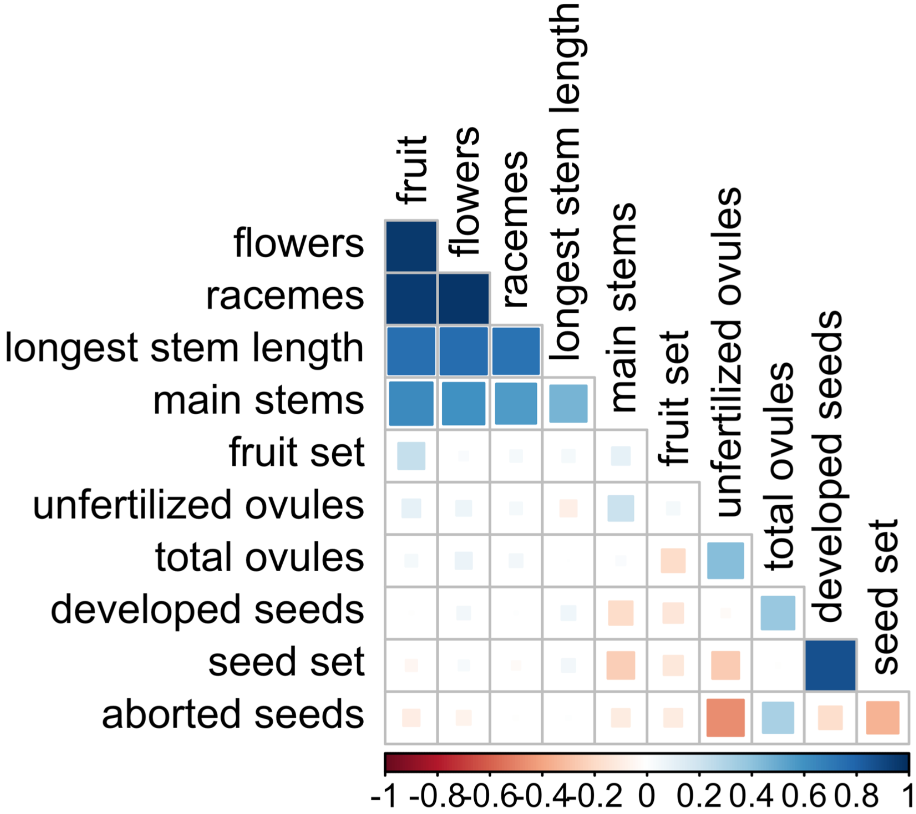

Figure 8. Correlation matrix of plant size and reproductive success variables measured in 2019. Fruit, flowers, racemes, length of longest stem, and main stems, are the maximum number counted/measured per plant over the season. Unfertilized ovules, total ovules, fruit set, developed seeds, seed set, and aborted seeds are the average number counted per collected fruit. Fruit set is the percentage of flowers that became fruit and seed set is the percentage of total ovules that filled into developed seeds.

There was no effect of site on *A. microcymbus* reproduction (*P* = 0.40, SS = 21.7, MS = 10.85) (Tables 5 and 6). Length of longest stem had a significant effect on the additive absolute measures of reproduction including numbers of flowers (*P* <0.001, F-statistic = 39.57, R^2 = 0.33) and number of fruit (*P* < 0.001, F-statistic = 39.36, R^2 = 0.33), but no effect on fruit set (*P* = 0.63, F-statistic = 1.189, R^2 = 0.002) or seed set (*P* = 0.65, F-statistic = 0.2125, R^2 = -0.01) (Table 7).

Table 5. The coefficients of the ANOVA comparing reproduction across sites. Reproduction was the additive predictors maximum fruit, maximum flowers, maximum racemes, average total ovules per fruit, average total seeds per fruit, average fruit set, average seed set, average ratio of aborted seeds, and average ratio of undeveloped ovules.

Model components	DF	SS	MS	F	*P*-value
Site	2	21.7	10.85	0.941	0.40
residuals	77	888.0	11.53		

Table 6. Pairwise differences in reproduction across sites. There was no effect of site.

Site	diff	Lower CL	Upper CL	p- adjusted
DS1 – SBC	1.0168316	-1.582282	3.615945	0.6199728
DS2 – SBC	1.5616939	-1.162066	4.285453	0.3614631
DS2 – DS1	0.5448623	-1.465404	2.555129	0.7941951

Table 7. Model output of the relationship between the length of the longest stem and the number of flowers, the number of fruits, fruit set (the ratio of flowers that develop into fruit) and seed set (the ratio of ovules that develop into seeds).

Model	Estimate	Std. Error	t-value	p-value
Number of Flowers				
Intercept	-4321.6	901.1	-4.796	<0.001
Square root of max. height	796.5	126.6	6.290	<0.001
Residual standard error: 1143 on 78 degrees of freedom Multiple R^2: 0.34, Adjusted R^2: 0.33 F-statistic = 39.57 on 1 and 78 DF, p-value < 0.001				
Number of Fruit				
Intercept	-5188.6	1090.5	-4.758	< 0.001
Square root of max. height	961.3	153.2	6.273	< 0.001
Residual standard error: 1383 on 78 degrees of freedom Multiple R^2: 0.34, Adjusted R^2: 0.33 F-statistic: 39.36 on 1 and 78 DF, p-value: < 0.001				
Fruit Set				
Intercept	0.59990	0.08	6.708	< 0.001
Square root of max. height	-0.01371	0.01257	-1.091	0.28
Residual standard error: 0.1134 on 78 degrees of freedom Multiple R^2: 0.02, Adjusted R^2: 0.002 F-statistic: 1.189 on 1 and 78 DF, p-value: < 0.28				
Seed Set				
Intercept	0.45300	0.19810	2.287	0.02
Square root of max. height	0.01283	0.02784	0.461	0.65
Residual standard error: 0.2513 on 78 degrees of freedom Multiple R^2: 0.003, Adjusted R^2: -0.01 F-statistic: 0.2125 on 1 and 78 DF, p-value: < 0.65				

2020 Fruit Set-

The fruit to flower ratio, calculated from the combined total flowers counted and the combined total fruit counted was 0.24 at DS1, 0.23 at DS2, 0.10 at SBC, 0.07 at YU, and 0.04 at DF.

Duration of Flower Bloom and Fruit Maturation -

Open flowers were observed on plants from 2.5 - 4 days. No fruit were monitored from start until end, although fruit were observed on plants for up to at least 16 days, beginning from the period that they were visible inside the corolla.

2020 Herbivory-

From 19 June through 15 July across all sites and visits combined, the average length of the longest stems that were not grazed was 24 cm (SD = 12.58, SE = 1.16, *n*=118), while the average length of the longest stems that were grazed was 17 cm (SD = 16.89, SE = 9.95, *n* = 84) (Figure 9). During the fifth collection period the number of browsed stems bitten at a diagonal was 88% at DS1, 78% at DS2, 84% at SBC, 83% at YU, and 88% at DF.

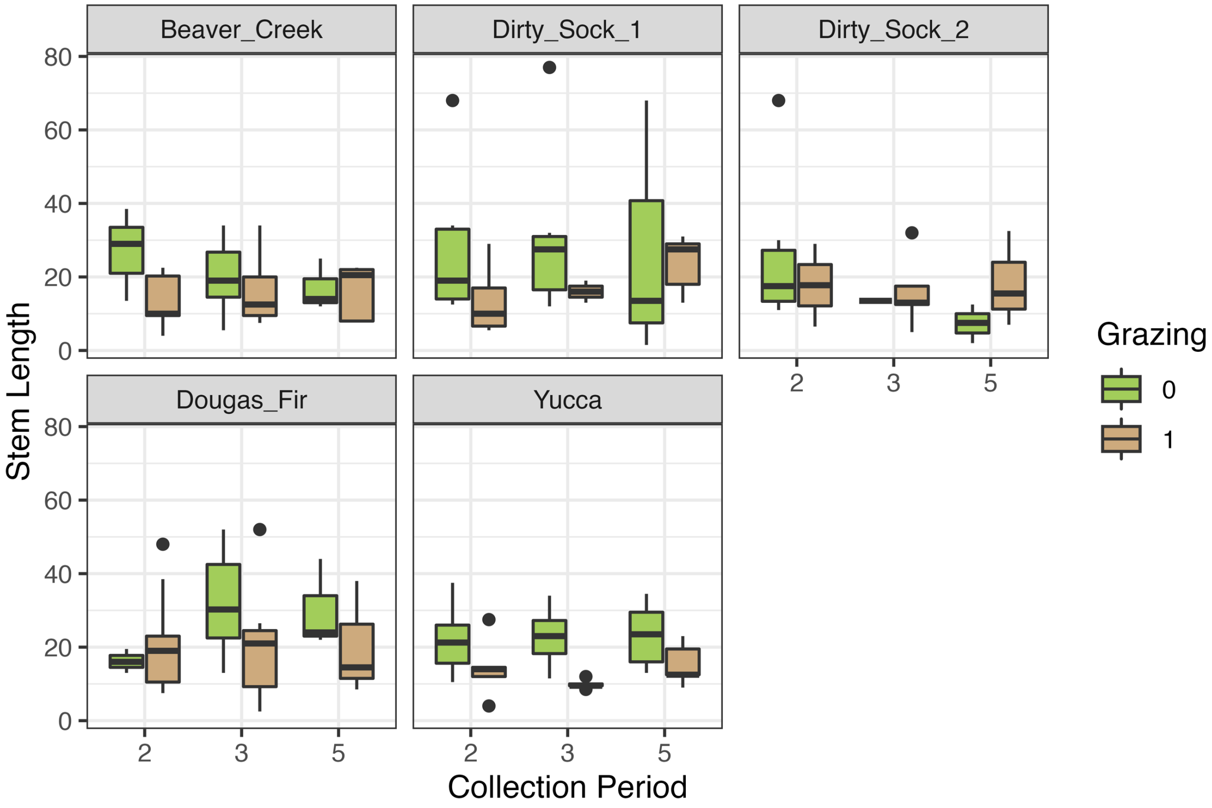

Figure 9. The length of longest stems measured across sites on plants from the period 19 June to 15 July. Intervals were 6 days apart. 0 = grazing absent from measured stem and 1 = grazing present on measured stem.

Across all sites combined, the average percentage of plants categorized as having high levels of herbivory was 36% during the first data collection period, 47% during the second, 77% during the third, and 84% during the fifth (there were no data collected during the fourth period). SBC and YU had lower herbivory during the earlier part of the season than other sites, with 23% and 40% of individuals showing high levels of herbivory by the second data collection, respectively. In contrast, by the second data collection, the number of individuals with high herbivory was 66% at DS1, 73% at DF and 80% at DS2. By the fifth data collection, at all sites most individuals had high levels of herbivory, with 90% at SBC, 67% at YU, 83% at DS1, 87% at DF and 96% at DS2 (Figure 10).

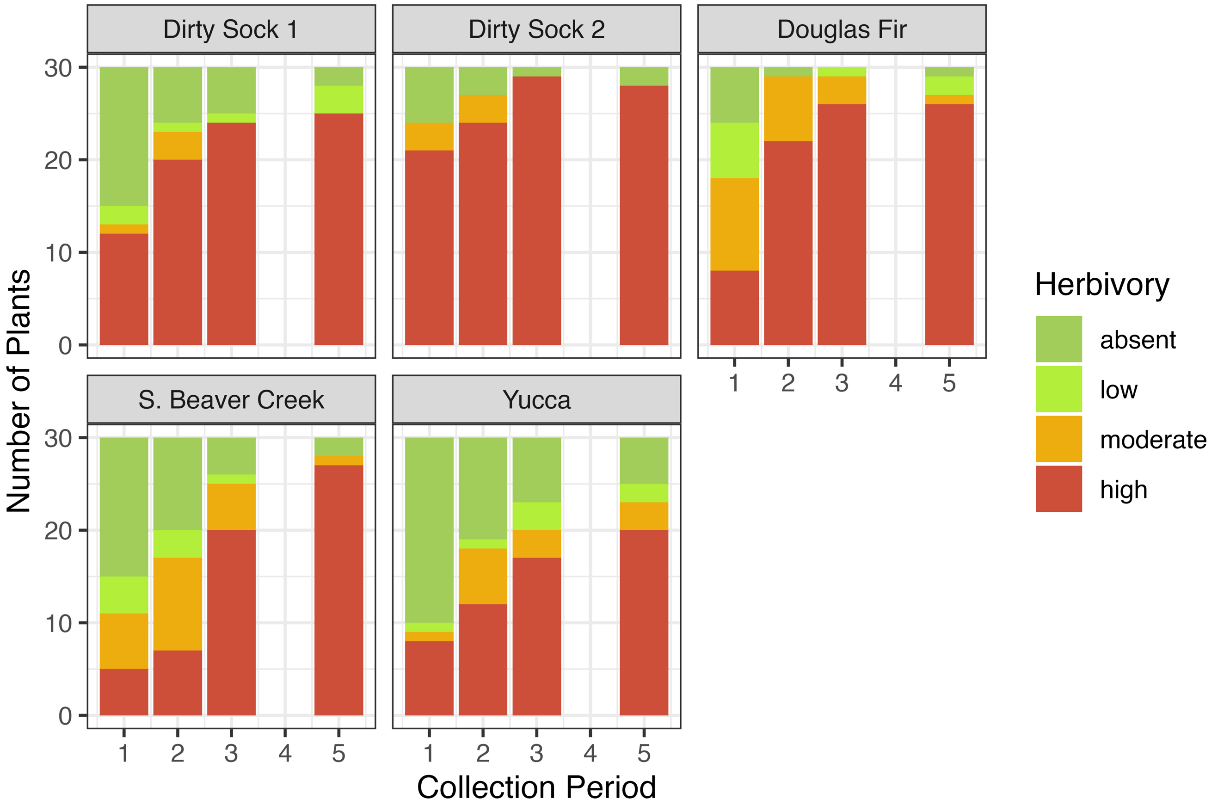

Figure 10. The degree of mammal herbivory observed on studied individuals from the period 19 June to 15 July. Intervals were 6 days apart.

Game Cameras-

Out of the three *A. microcymbus* plants photographed during the first two weeks of August, a cottontail rabbit was observed eating foliage at one plant on 8 August around 8:30pm at SBC (Figure 11). The grazed stems were at a 45-degree angle when I collected the camera.

Figure 11. A mountain cottontail rabbit observed grazing an *A. microcymbus* plant on 8 August, around 8:30 pm at the SBC site. In this photo, the plant is located at the base of the sagebrush, and a branch is in the rabbit's mouth.

Figure 12. Small mammal herbivory on an *A. microcymbus* plant.

Pollination Ecology

Breeding System Study-

Due to dry growing conditions and the impacts of herbivory, results were only obtained from 5 out of the 90 pollinator exclusion bags that were placed out in 2020, and 10 results were obtained out of the 44 pollinator exclusion bags placed out in 2021 (Table 8). The three developed seeds collected from this study were tested for viability and were determined to be viable.

Table 8. The results of the 2020 and 2021 pollinator exclusion experiment.

Site	Year	# of flowers	# of fruit	# of seeds	fruit/flower ratio
DS1	2020	2	0		0
DS1	2020	18	0		0
SBC	2020	2	0		0
SBC	2020	6	1 aborted	1 aborted	0
SBC	2020	2	0		0
YU	2021	15	1	2	0.06
YU	2021	13	1	1	0.08
YU	2021	13	0		0
YU	2021	28	0		0
YU	2021	29	1	1 aborted	0
YU	2021	7	0		0
YU	2021	19	0		0
YU	2021	18	0	N/A	0
DS1	2021	9	0	N/A	0
DS1	2021	13	0	N/A	0

Insect Visitors-

Solitary bees including *Ashmeadiella sculleni, Ashmeadiella lutzi, Ashmeadiella cactorum* and *Anthidium emarginatum* were observed eliciting the pollination mechanisms of *A. microcymbus.* These bees often approached flowers from the front and landed, pressing the keel downwards and accessing the reproductive structures. In addition to these insect visitors, two species of solitary bees not tripping the keel were identified, including *Ceratina nanula* and

Lasioglossum sp. *Ceratina nanula* was observed inserting its glossa into the side of the flower where the wing petals connect to the banner petals. *Lasioglossum* sp. was observed inserting its glossa into the back of the flower where the wing petals connect to the banner petals and also into the back of the flower where the banner petal connects to the sepals. On one occasion, a *Lasioglossum* sp. was observed puncturing a hole in the back of the sepals. Neither of these species were observed opening the keel and directly accessing the anthers. In addition to these Hymenoptera, other visitors that did not open the keel included two species of Lepidoptera including *Plebejus melissa* (Melissa blue butterfly), micromoths (collected but not identified), and a species of Diptera in the genus *Geron. Bombus* sp. were also observed in 2019 and 2020. Collections and identification of *Bombus* sp. were not made because careful behavior observations were not part of the protocol in 2019 and I did not capture the single individual I observed in 2020. During the course of this study, a total of 13 Hymenoptera, 7 Lepidoptera and 1 Diptera were captured and mounted for identification. Specimens were stored in the Western Colorado University Faunal Museum in Gunnison, CO (including 3 *Ashmeadiella* sp., 1 *Anthidium* sp., and 3 *Plebejus melissa*) and the USDA ARS U.S. National Pollinating Insects Collection in Logan, UT (including 1 *Ashmeadiella sculleni,* 2 *Ashmeadiella lutzi*, 2 *Anthidium emarginatum*, 4 *Ashmeadiella cactorum*, 2 *Lassioglossum* sp., 1 *Ceratina nanula,* and 1 *Geron* sp.).

Figure 13. *Ashmeadiella* sp.

Figure 14. *Ashmeadiella* sp.

Figure 15. *Anthidium* sp.

Post-Primary Dispersal Seed Ecology

Soil Seed Bank Characteristics-

All seeds were found in the 16 meshes per inch standard mesh soil sieve. During November 2020, the average number of seeds found across all sites combined was 1.80 (SD = 2.08, SE = 0.29, *n* = 50) at 0.0 meters, 0.64 (SD = 1.16, SE = 0.16, *n* = 50) at 0.5 meters, and 0.24 (SD = 0.55, SE = 0.08, *n* = 50) at 1.0 meters (Table 9, Figure 16).

Table 9. The average number of seeds found at 0.0, 0.5, and 1.0 meters from the base of the plant at each site in November 2020. At each site and distance, *n* = 10.

Site	Number of seeds found at 0.0 meters	Number of seeds found at 0.5 meters	Number of seeds found at 1.0 meter
DS1	1.1 (SD = 1.29, SE = 0.41)	0.5 (SD = 1.27, SE = 0.40)	0.2 (SD = 0.63, SE = 0.2)
DS2	1.4 (SD = 1.84, SE = 0.58)	0.4 (SD = 0.70, SE = 0.22)	0.4 (SD = 0.70, SE = 0.22)
SBC	2.2 (SD = 1.75, SE = 0.55)	0.6 (SD = 0.97, SE = 0.31)	0.2 (SD = 0.42, SE = 0.13)
YU	3.3 (SD = 3.06, SE = 0.97)	0.9 (SD = 1.20, SE = 0.38)	0.4 (SD = 0.70, SE = 0.22)
DF	1.0 (SD = 1.41, SE = 0.45)	0.8 (SD = 1.62, SE = 0.51)	0 (SD = 0, SE= 0)

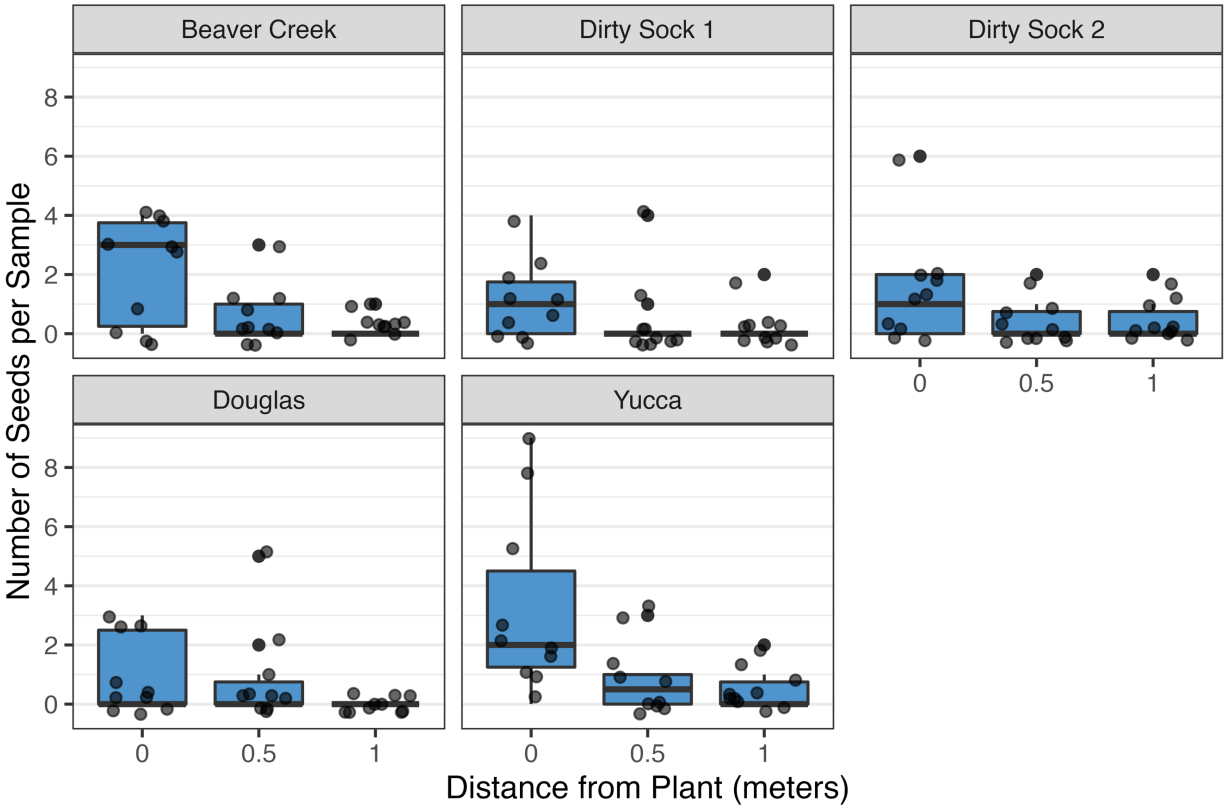

Figure 16. The number of seeds found per soil sample (395.84 cubic centimeters) at 0 m, 0.5 m, and 1.0 m from the focal plant.

Fewer seeds were found in soil samples taken with increasing distance from the focal plant. There was a posterior probability greater than 95% that the effect of distance on the number of seeds was less than 0 (Equation 2, Table 10). Based on this model, there was an expected decrease of 86.5 % in the number of seeds found from the base of the focal plant to 1.0 meter from the focal plant. From 0.0 meters to 0.5 meters, there was an expected decrease of 65.5 %, and from 0.5 meters to 1.0 meter, there was an expected decrease of 61%. The predicted number of seeds was 1.71 at 0.0 meters from the parent plant, 0.59 at 0.5 meters, and 0.23 at 1.0.

Table 10. The estimated parameter and uncertainty for the linear mixed effects model between number of seeds and distance from plant. Standard deviations are of point estimates of group effects.

Parameter	Mean	Lower 5 % CI	Upper 95 % CI	Effective sample size
β_0 (0.0 meters from plant)	0.53	0.087	0.97	5588
β_1 (0.5 meters from plant)	-1.06	-1.57	-0.54	10519
β_2 (1.0 meter from plant)	-2.02	-2.68	-1.39	10695
Standard deviation (plant I.D. by intercept)	0.12	0.0004	0.44	2943
Standard deviation (site by intercept)	0.18	0.0007	0.71	3577

At 0.5 meters from the base of the parent plant, there were fewer seeds in sites with higher proportion bare ground. At 0.0 and 1.0 meter from the parent plant, there was not a credible effect of bare ground cover on the number of seeds found per sample (posterior credible interval 95%). At 0.5 meters from the parent plant, there were more seeds found in microhabitat types with less bare ground. There was a posterior probability greater than 95% that the interaction between bare ground and distance from plant at 0.5 meters was less than 0 (95% posterior credible interval, -0.0376 to -0.0015) (Equation 3, Table 11, Figure 17).

At 0.0 meters from the parent plant, the predicted number of seeds was 1.54 at 0% bare ground, 1.71 at 50% bare ground and 1.41 at 100% bare ground. At 0.5 meters from the parent plant, the predicted number of seeds was 1.55 at 0% bare ground, 0.65 at 50% bare ground and 0.28 at 100% bare ground. At 1.0 meters from the parent plant, the predicted number of seeds was 0.17 at 0% bare ground, 0.21 at 50% bare ground and 0.25 at 100% bare ground.

Table 11. The estimated parameter and uncertainty for the linear mixed effects model for the relationship between number of seeds and distance from plant by bare ground exposure. Standard deviations are of point estimates of group effects.

Parameter	Mean	Lower 5% CI	Upper 95 % CI	Effective sample size
β_0 (0.0 meters from plant)	0.43	-0.26	1.13	7707
β_1 (0.5 meters from plant)	0.01	-1.08	1.105	8958
β_2 (1.0 meter from plant)	-2.24	-3.91	-0.72	9246
β_3 (bare ground)	0.0021	-0.009	0.013	9399
β_4 (0.5 meters from plant by bare ground)	-0.02	-0.04	-0.002	8280
β_5 (1.0 meter from plant by bare ground)	0.002	0-0.02	0.02	8446
Standard deviation (plant I.D. by site by intercept)	0.13	0.0004	0.47	4944
Standard deviation (site by intercept)	0.16	0.0005	0.601	6919

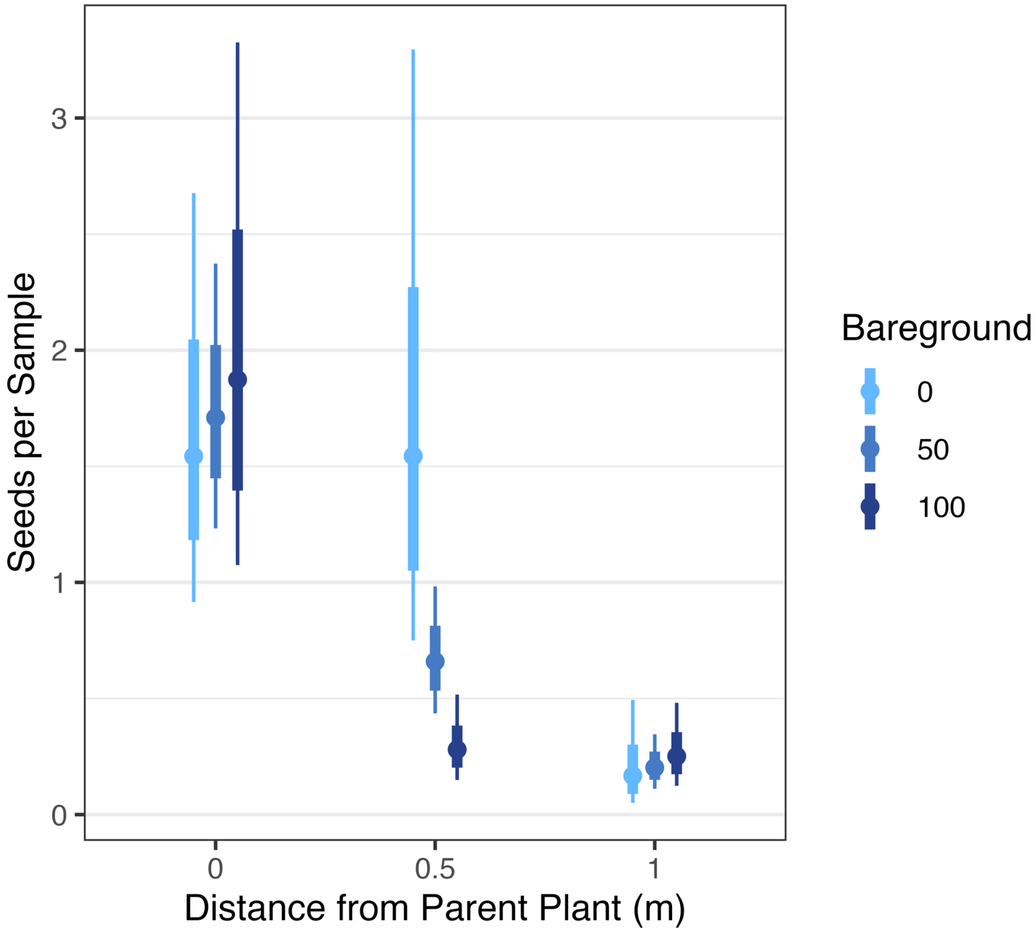

Figure 17. The estimated number of seeds at microsites with 0%, 50%, and 100% bare ground exposure for each distance measured from parent plant.

The effect of bare ground remained credible when rocks and vegetation were included in this model, but rocks and vegetation were not credible when tested with bare ground (Equations 6 and 7). When the number of seeds was modeled by the interaction of distance and rocks alone (Equation 4), at 0.5 meters from the parent plant, there were more seeds found in microhabitat types with less bare ground. There was a posterior probability greater than 90% that the interaction between rock cover and distance from plant at 0.5 m was greater than 0 (90% posterior credible interval, 0.0034-0.0576) (Table 12, Figure 18). Vegetation cover alone was not a significant predictor of seed count (Equation 5), but there is a visual positive trend showing an association between vegetation cover and more seeds at 0.5 meters (Table 13, Figure 19). These trends show that more seeds are found in area with higher percent cover of non-bare ground structures (rocks and vegetation), however it is unclear which combination of non-bare ground structures account for higher seed counts.

Table 12. The estimated parameters and uncertainty for the linear mixed effects model showing the relationship between number of seeds and distance from plant by rock cover. Standard deviations are of point estimates of group effects.

Parameter	Mean	Lower 10% CI	Upper 90 % CI	Effective sample size
β_0 (0.0 meters from plant)	0.54	0.17	0.90	5170
β_1 (0.5 meters from plant)	-1.52	-2.06	-0.99	15776
β_2 (1.0 meters from plant)	-1.87	-2.47	-1.29	17187
β_3 (rock cover)	-0.0005	-0.02	0.02	12905
β_4 (0.5 meters from plant by rock)	0.03	0.003	0.06	11968
β_5 (0.5 meters from plant by rock)	-0.02	-0.06	0.02	13102
Standard deviation (plant I.D by site by intercept)	0.10	0.002	0.28	6882
Standard deviation (site by intercept)	0.20	0.004	0.47	4403

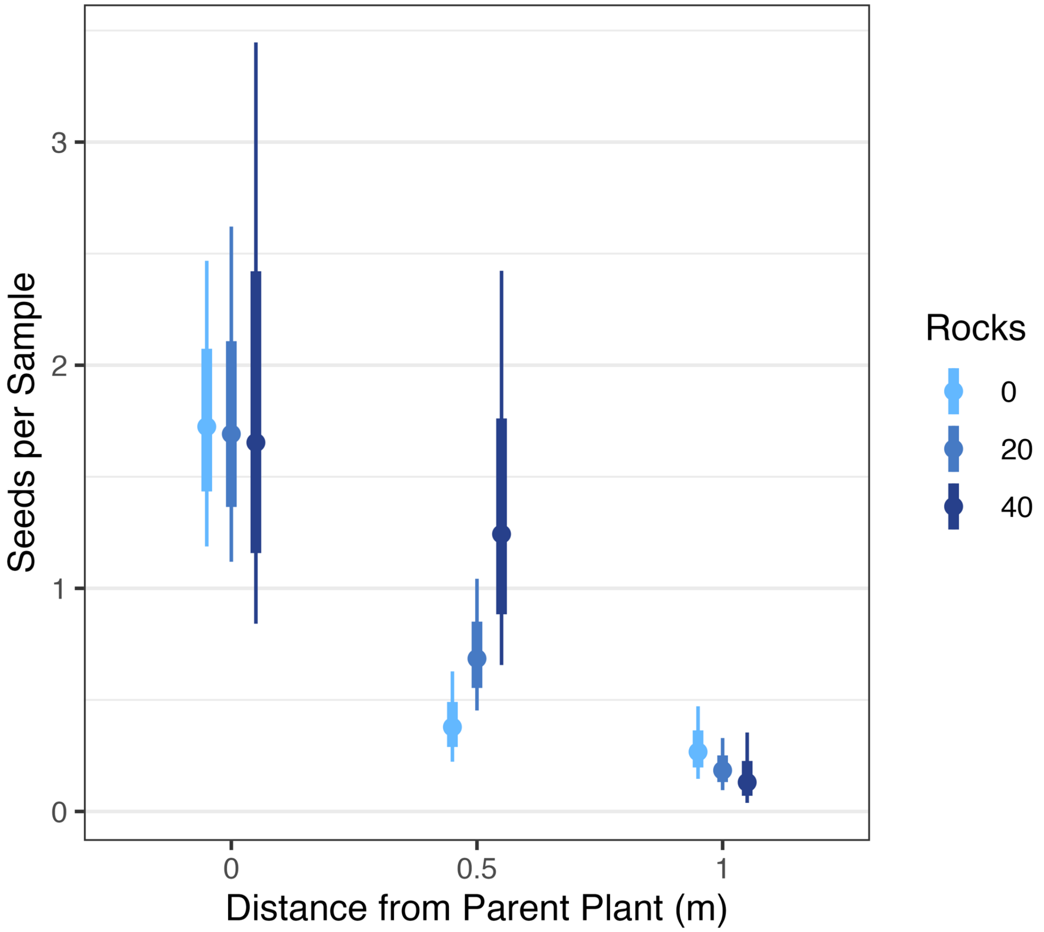

Figure 18. The predicted number of seeds for the estimated low, median, and high values of rock cover.

Table 13. The estimated parameters and uncertainty for the linear mixed effects model showing the relationship between number of seeds and distance from plant by vegetation cover. Standard deviations are of point estimates of group effects.

Parameter	Mean	Lower 0.10	Upper 0.90	Effective sample size
β_0 (0.0 meters from plant)	0.55	-0.01	1.10	7913
β_1(0.5 meters from plant)	-1.41	-2.09	-0.75	9190
β_2(1.0 meter from plant)	-2.15	-2.89	-1.41	9856
β_3 (vegetation)	-0.0002	-0.008	0.007	8664
β_4 (0.5 meters from plant by vegetation)	0.01	-0.002	0.02	9554
β_5 (1.0 meter from plant by vegetation)	0.003	-0.01	0.02	11161
Standard deviation (plant I.D. by site by intercept)	0.15	0.002	0.39	4859
Standard deviation (site by intercept)	0.16	0.002	0.38	8737

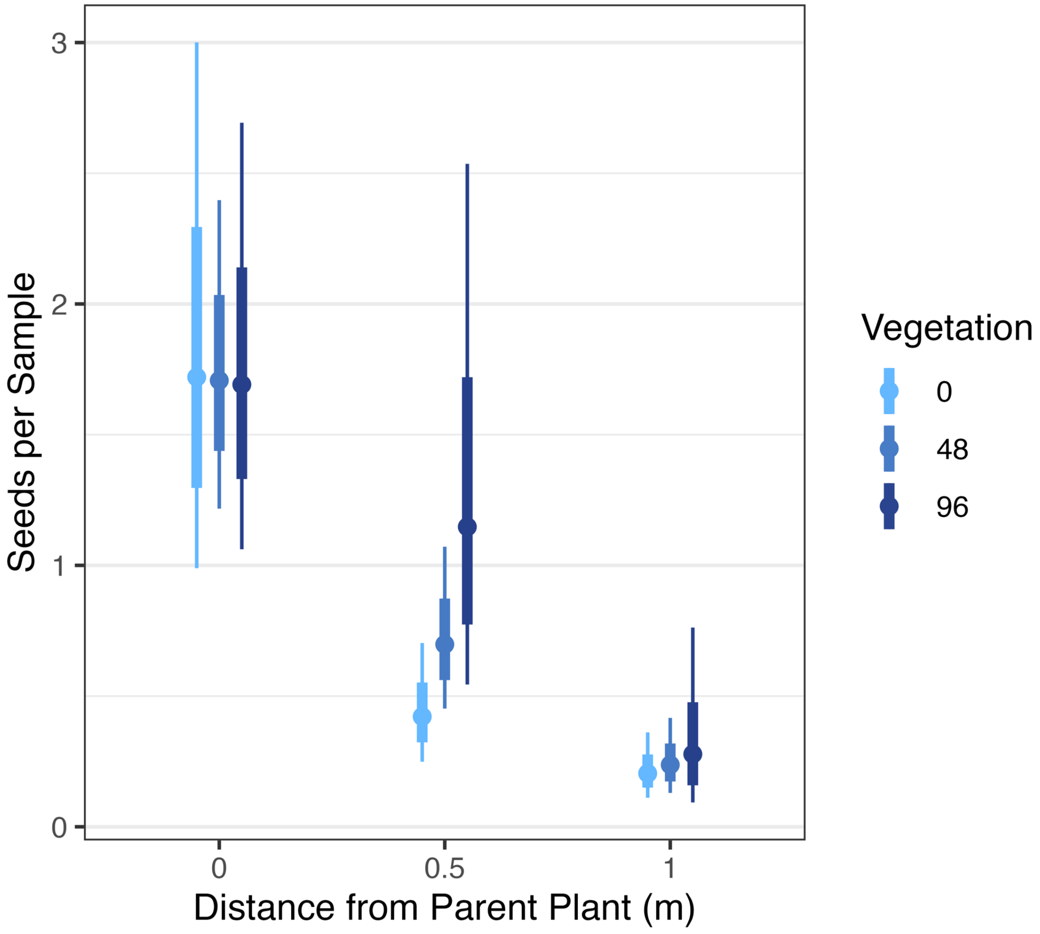

Figure 19. The predicted number of seeds for the for the estimated low, median, and high values of vegetation cover.

Seed Viability-

The combined total percent of viable seeds retrieved from soil samples across all three sites was 81%. The average percent of viable seeds was 78% at DS1, 71% at DS2, 87% at South Beaver Creek, 89% at Douglas Fir and 81% at Yucca.

Seeds Over Time-

During November 2019, the average number of seeds found at YU was 6.4 (SD = 8.33, SE = 2.63, *n* = 10) at 0.0 m, 2.6 (SD = 4.03, SE = 1.28, *n* = 10) at 0.5 m, and 1.7 (SD = 4.35, SE = 1.37, *n* = 10) at 1 m. During June 2019, the average number of seeds found at YU was 4.6 (SD = 6.65, SE = 2.10, *n* = 10) at 0.0 m, 1.3 (SD = 0.95, SE = 0.3, *n* = 10) at 0.5 m, and 0.9 (SD = 0.99, SE = 0.31, *n* = 10) at 1 m (Figure 20). November 2020 averages are reported above. The effect of collection period on number of seeds was not tested because sampling criteria differed between preliminary samples (November 2019 and June 2020) and November 2020 samples.

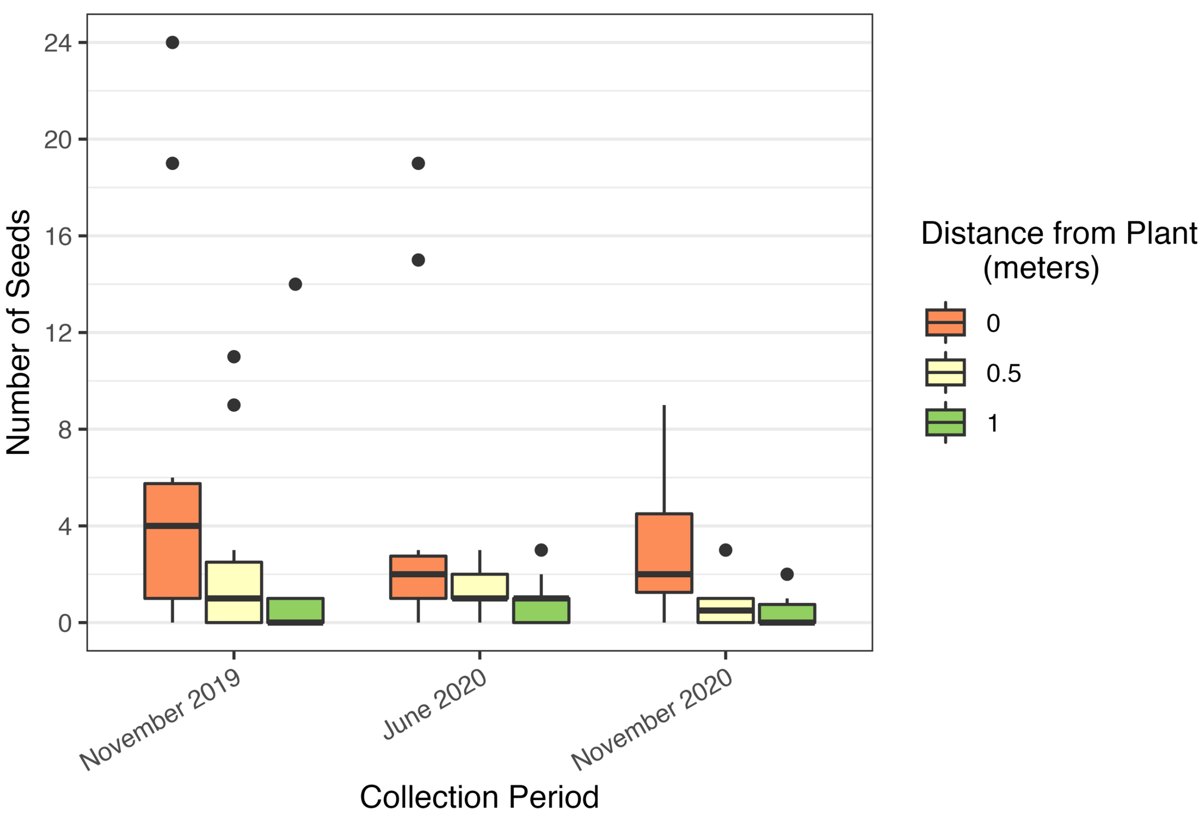

Figure 20. The number of seeds found per soil sample (395.84 cubic centimeters) at 0 m, 0.5 m, and 1.0 m from the focal plant, from November 2019 to November 2020 at YU site.

Microhabitat Characteristics of Established Plants-

Astragalus microcymbus individuals were found growing in locations closer to vegetation patches than random points distributed across the landscape in 2020 ($P = 0.03$, $X^2 = 103.14$, DF = 78) and 2021 ($P = 0.02$, $X^2 = 68.538$, DF = 48) (Figure 21). There was no difference in distance between random points and *A. microcymbus* plants to the nearest rock cover (cobbles and boulders) (Figure 22), *A. tridentata* (including live or dead), or live *A. tridentata*.

2020

Table 14. Average distance (cm) from random points and *A. microcymbus* plants to the nearest rock. For each average, *n* = 25.

Site	Distance to Random Point	Distance to *A. microcymbus*
Nearest Rock		
DS1	7.3 (SD = 11.0, SE = 2.2)	11.3 (SD = 11.76, SE = 2.35)
DS2	8.9 (SD = 8.25, SE = 1.65)	6.9 (SD = 8.16, SE = 1.63)
SBC	18.1 (SD = 18.43, SE = 3.69)	19 (SD = 14.36, SE = 2.87)
DF	6.3 (SD = 6.17, SE = 1.23)	4.8 (SD = 3.82, SE = 0.76)
Nearest Vegetation		
DS1	4.5 (SD = 4.73, SE = 0.95)	2.4 (SD = 5.44, SE = 1.09)
DS2	4.3 (SD = 4.30, SE = 4.44)	2.6 (SD = 5.59, SE = 1.12)
SBC	7.1 (SD = 5.99, SE = 1.20)	1.4 (SD = 2.60, SE = 0.52)
DF	6.0 (SD = 5.14, SE = 1.03)	1.6 (SD = 2.28, SE = 0.46)
Nearest *A. tridentata* (including dead)		
DS1		
DS2	0.74 (SD = 0.37, SE = 0.07)	0.72 (SD = 0.50, SE = 0.10)
SBC	0.73 (SD = 0.49, SE = 0.10)	0.70 (SD = 0.54, SE = 0.11)
DF	1.14 (SD = 0.87, SE = 0.17)	0.90 (SD = 0.83, SE = 0.17)
YU	0.64 (SD = 0.40, SE = 0.08)	0.80 (SD = 0.43, SE = 0.09)
	1.13 (SD = 0.79, SE = 0.16)	1.14 (SD = 0.78, SE = 0.16)
Nearest live *A. tridentata*		
DS1	0.98 (SD = 0.50, SE = 0.10)	0.89 (SD = 0.53, SE = 0.11)
DS2	0.88 (SD = 0.56, SE = 0.11)	0.87 (SD = 0.63, SE = 0.13)
SBC	1.20 (SD = 0.86, SE = 0.17)	0.97 (SD = 0.87, SE = 0.17)
DF	0.78 (SD = 0.56, SE = 0.11)	1.05 (SD = 0.71, SE = 0.14)
YU	1.38 (SD = 0.97, SE = 0.19)	1.38 (SD = 0.83, SE = 0.17)

2021

Table 15. Average distance (cm) from random points and *A. microcymbus* to the nearest rock and nearest vegetation at each site. For each average, *n* = 25.

Site	Distance to Random Point	Distance to *A. microcymbus*
Nearest Rock		
DS1	11.9 (SD = 9.47, SE = 1.90)	10.66 (SD = 7.02, SE = 1.4)
DS2	8.9 (SD = 10.32, SE = 2.06)	8.12 (SD = 9.13, SE = 1.83)
SBC	25.66 (SD = 31.32, SE = 6.26)	30.58 (SD = 18.33, SE = 3.67)
YU	8.7 (SD = 9.02, SE = 1.80)	9.8 (SD = 8.98, SE = 1.80)
Nearest Vegetation		
DS1	7 (SD = 6.82, SE = 1.36)	1.7 (SD = 4.88, SE = 0.98)
DS2	6.8 (SD = 7.26, SE = 1.45)	2.8 (SD = 4.80, SE = 0.96)
SBC	9.0 (SD = 6.29, SE = 1.26)	2.5 (SD = 3.35, SE = 0.67)
YU	9.7 (SD = 10.43, SE = 2.07)	2.6 (SD = 4.35, SE = 0.87)

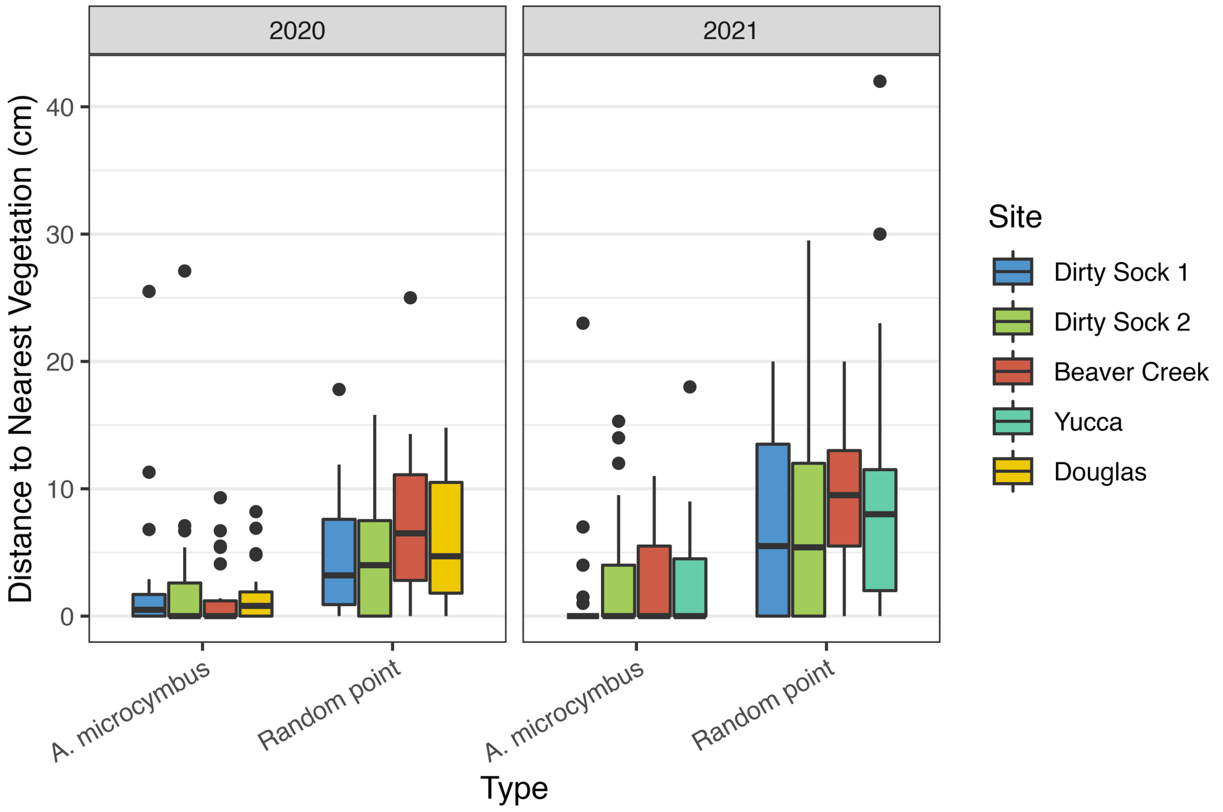

Figure 21. The distance to the nearest vegetation from random points and *A. microcymbus* plants across sites in 2020 and 2021.

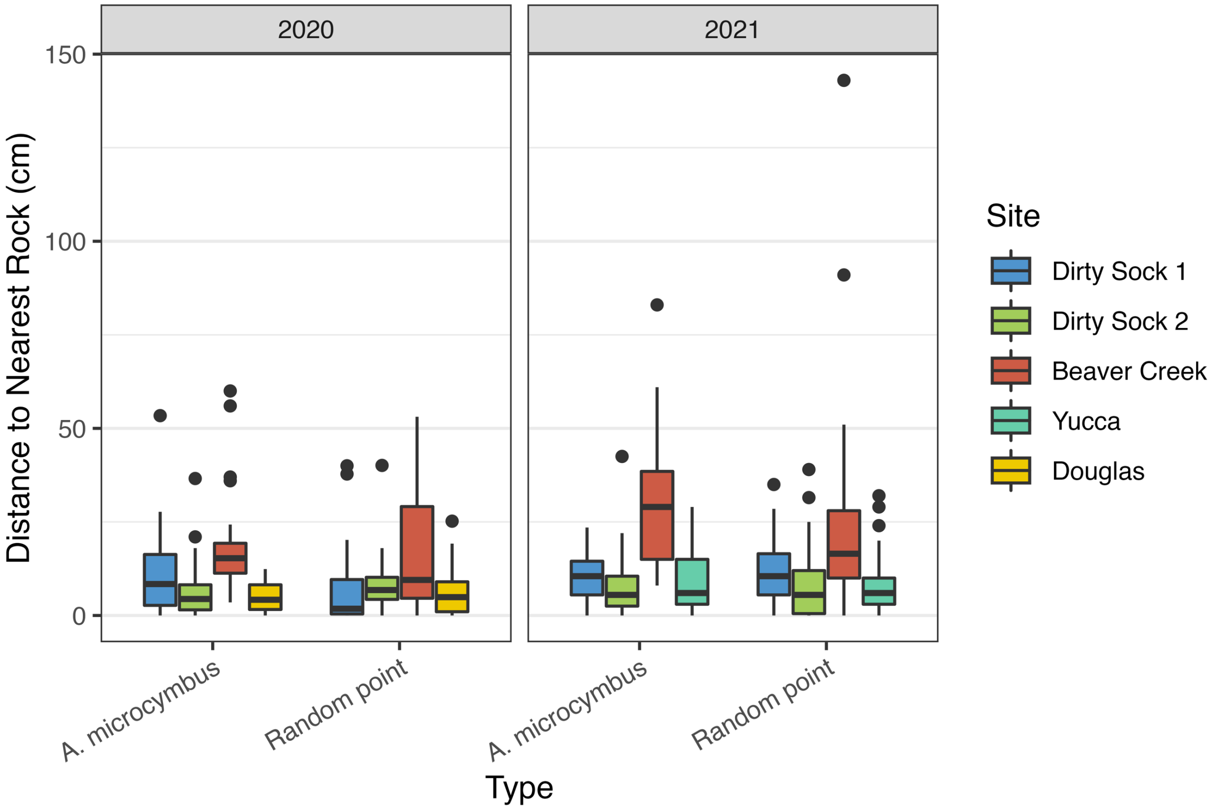

Figure 22. The distance to the nearest rock from random points and *A. microcymbus* plants across sites in 2020 and 2021.

Microhabitat composition also differed between random points and *A. microcymbus* plants. There was no effect of site on the difference in microhabitat composition cover during either 2020 or 2021 (Table 16 and 17). In both years that data were collected, percent exposure of bare ground was significantly lower and percent cover of vegetation was higher where *A. microcymbus* plants grew than at random points distributed across the landscape. There was no difference in percent cover of rock between *A. microcymbus* plants and random points (Table 18 and 19, Figure 23).

Table 16. The coefficients of the ANOVA of the effect of site, by type (random point or *A. microcymbus* plant) and microhabitat percent cover in 2020. Percent cover of microhabitat types differed significantly between random points and *A. microcymbus* plants. The interaction between type and microhabitat are significant at the <0.05 interval. Microhabitat alone and the interaction of microhabitat and site are also statistically significant although these are not ecologically important predictors on their own, without the inclusion of the predictor type (random point or *A. microcymbus* plant).

Predictor	DF	SS	MS	F	p-value
Type	1	319	319	0.247	0.62
Microhabitat	2	345989	172994	134.189	<0.001
Site	4	1643	411	0.319	0.87
Type by microhabitat cover	2	99256	49628	38.496	<0.001
Point type by site	4	1109	277	0.215	0.93
Microhabitat by site	8	22269	2784	2.159	0.03
Type by microhabitat cover by site	8	10338	1292	1.002	0.43
Residuals	720	928213	1289		

Table 17. The coefficients of the ANOVA of the effect of site, point type (random point or *A. microcymbus* plant) and microhabitat percent cover in 2021. Percent cover of microhabitat types differed significantly between random points and *A. microcymbus* plants. Microhabitat alone and the interaction of microhabitat and site are also statistically significant although these are not ecologically important predictors on their own, without the inclusion of the predictor point type (random point or *A. microcymbus* plant).

Predictor	DF	SS	MS	F	p-value
Type	1	287	278	0.186	0.67
Microhabitat	2	244373	122187	79.151	<0.001
Site	3	197	66	0.043	0.99
Type by microhabitat cover	2	72274	36137	23.409	<0.001
Type by site	3	335	112	0.072	0.96
Microhabitat by site	6	22864	3811	2.468	0.02
Type by microhabitat cover by site	6	13412	2235	1.448	0.19
Residuals	573	884548	1544		

Table 18. The pairwise differences in microhabitat cover between random points and *Astragalus microcymbus* plants during 2020.

Point Type	Microhabitat Type	Marginal estimated mean	SE	df	Lower CL	Upper CL
Random point	Bare ground	66.28	2.92	300	60.539	72.0
A. microcymbus		41.03	2.92	300	35.291	46.8
Random point	Rocks	8.40	2.92	300	2.656	14.1
A. microcymbus		6.43	2.92	300	0.691	12.2
random point	Vegetation	36.86	2.92	300	31.123	42.6
A. microcymbus		67.84	2.92	300	62.099	73.6

Table 19. The pairwise differences in microhabitat cover between random points and *Astragalus microcymbus* plants during 2021.

Point Type	Microhabitat Type	Marginal estimated mean	SE	df	Lower CL	Upper CL
Random point	Bare ground	61.47	3.93	573	53.75	69.2
A. microcymbus		43.19	3.95	573	35.43	50.9
Random point	Rocks	13.82	3.93	573	6.10	21.5
A. microcymbus		4.23	3.95	573	-3.53	12.0
random point	Vegetation	35.68	3.93	573	27.96	43.4
A. microcymbus		67.69	3.95	573	59.94	75.5

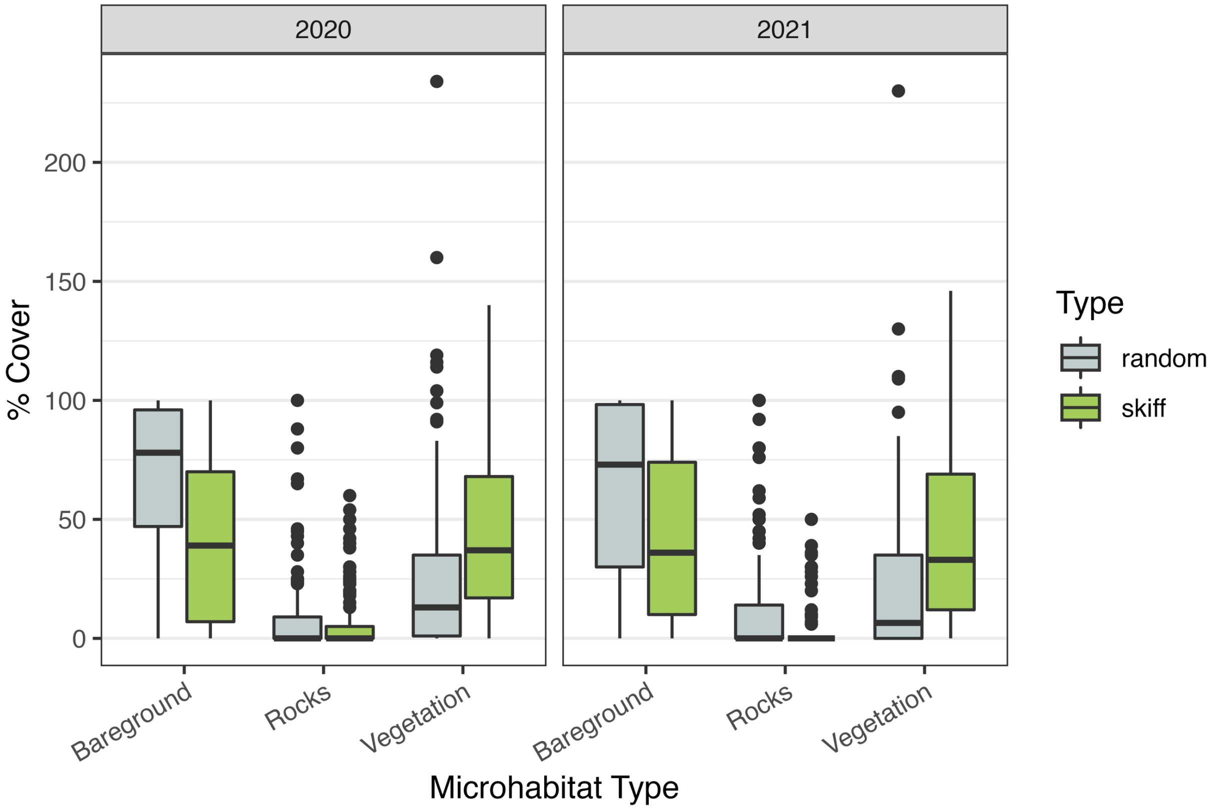

Figure 23. Distribution of percent cover values estimated for bare ground, rocks and vegetation at random points and *A. microcymbus* plants studied both years.

2020

Table 20. Average percent exposure of bare ground, and percent cover of rocks and vegetation per site at random points and *A. microcymbus* plants in 2020. For all averages, $n = 25$.

Site	Random Point	*A. microcymbus*
Bare ground		
DS1	62% (SD = 29.6, SE = 5.92)	39% (SD = 38.7, SE = 32.9)
DS2	62% (SD = 37.4, SE = 7.47)	40% (SD = 33.2, SE = 6.64)
SBC	79% (SD = 32.5, SE = 6.49)	46% (SD = 39.2, SE = 7.85)
DF	65% (SD = 31.4, SE = 6.28)	45% (SD = 31, SE = 6.20)
YU	62% (SD = 36.2, SE = 7.23)	36% (SD = 34.3, SE = 6.87)
Rocks		
DS1	14% SD = 21.9, SE = 4.37)	5% (SD = 13.3, SE = 2.67)
DS2	3 % (SD = 6.72, SE = 1.34)	8% (SD = 12.6, SE = 2.51)
SBC	3% (SD = 13.4, SE = 2.69)	0.2% (SD = 0.8, SE = 0.16)
DF	15% (SD = 14.7, SE = 4.73)	11% (SD = 14.5, SE = 2.91)
YU	8% (SD = 18.1, SE = 3.63)	8% (SD = 15.9, SE = 3.18)
Vegetation		
DS1	31% (SD = 39.3, SE = 7.86)	64% (SD = 39.3, SE = 7.87)
DS2	51% (SD = 59.7, SE =11.9)	70% (SD = 47.2, SE = 9.43)
SBC	27% (SD = 46.7, SE = 9.35)	79% (SD = 56.2, SE = 11.2)
DF	26% (SD = 30.5, SE = 6.11)	56% (SD = 41.0, SE = 8.20)
YU	50% (SD = 72.9, SE = 14.6)	70% (SD = 51.4, SE = 10.3)

2021

Table 21. Average percent exposure of bare ground, and percent cover of rocks and vegetation per site at random points and *A. microcymbus* plants in 2021. For *A. microcymbus* and random points at SBC, *n* = 24. For other averages, *n* = 25.

Site	Random Point	*A. microcymbus*
Bare ground		
DS1	60% (SD = 40.2, SE = 8.04)	27% (SD = 30.1, SE = 6)
DS2	53% (SD = 37.9, SE = 7.59)	52% (SD = 32.0, SE = 6.40)
SBC	70% (SD = 41.3, SE = 8.26)	53% (SD = 35.7, SE = 7.30)
YU	63% (SD = 34.3, SE = 6.86)	41% (SD = 35.1, SE = 7.01)
Rocks		
DS1	9% (SD = 23.1, SE = 4.63)	4% (SD = 12.0, SE = 2.40)
DS2	21% (SD = 31.5, SE = 6.29)	6% (SD = 10.3, SE = 2.07)
SBC	9% (SD = 25.7, SE = 5.14)	0% (SD = 0, SE = 0)
YU	17% (SD = 17, SE = 4.63)	7% (SD = 12.6, SE = 2.51)
Vegetation		
DS1	43% (SD = 54.8, SE = 11)	87% (SD = 52.2, SE = 10.4)
DS2	36% (SD = 57.0, SE = 11.4)	54% (SD = 35.3, SE = 7.06)
SBC	35% (SD = 69.4, SE = 13.9)	60% (SD = 60.0, SE = 12.2)
YU	29% (SD = 50.8, SE =10.2)	70% (SD = 48.3, SE = 9.67)

DISCUSSION

Floral diversity is being threatened globally, facing challenges of habitat loss, climate change, and over exploitation (Antonelli et al. 2020). This loss of species threatens ecosystem resilience and function, and irreversibly erases unique evolutionary heritage (Antonelli et al. 2020, Hooper et al. 2005, Neely et al. 2009). A unique component of the planet's biodiversity occurs as endemics, however due to limited abundance and narrow distribution, these species may be especially vulnerable to genetic and environmental challenges (Barrett and Kohn 1991, Munson and Sher 2015, Neely et al. 2009). *Astragalus microcymbus,* a rare endemic to the sagebrush steppe of Gunnison and Saguache counties in western Colorado, is facing multiple growing pressures. An understanding of the reproductive ecology of this plant can help us understand these threats and if there are conservation efforts that can be taken to support its persistence.

This study set out to describe the reproductive success, pollination ecology and post-primary dispersal seed ecology of *A. microcymbus* guided by the following questions: 1.) What is the reproductive success of *A. microcymbus* as measured by fruit to flower and seed to ovule ratios? Does reproduction vary across sites and what factors influence its reproductive success? 2.) Does *A. microcymbus* require pollinators for successful seed set? If so, what are its primary pollinators? 3.) Does *A. microcymbus* have a viable soil seed bank and how does it vary across the landscape? Can insight into the secondary dispersal mechanisms and occurrence patterns of *A. microcymbus* be gained through the distribution of the seed bank and established individuals?

Astragalus microcymbus exhibits high year to year variation in reproduction. Even during a mast year as was observed in 2019, fairly low reproductive success was estimated. In 2020, low reproduction was observed due to the impacts of small mammal herbivory and drought, which also limited reproductive success study and the pollinator exclusion experiment. Solitary bees were observed visiting *A. microcymbus* and these may be important pollinators of this species. *Astragalus microcymbus* may exhibit some degree of a persistent soil seed bank and final seed fate may be influenced by both the processes of overland flow and the surrounding community vegetation structure.

Reproductive Success

The amount of reproduction was markedly different from 2019 to 2020. A pattern of high year to year variation has also been recorded in an ongoing long-term demographic study of *A. microcymbus* by DBG which found that year to year fruit production varies greatly, driven primarily by climatic factors (DePrenger-Levin and Hufft 2019). Fruit production in *A. microcymbus* is positively correlated with the number of above ground and reproductive individuals, indicating synchronism that is characteristic of masting (DePrenger-Levin and Hufft 2019). Resources may limit the frequency that populations can produce mast years through various ways. Resource constraints may come from varying interannual resource availability associated with environmental regimes (Pearse et al. 2016). Alternatively, plants may store resources until they have reached a sufficient amount to support a mast year, that once expended, are not available again until plants have reaccumulated these resources a following year (Pearse et al. 2016). The causes for the decrease in reproduction observed from 2019 to 2020 may be due in part to the natural interannual cycles of this plant's reproduction, although drought and herbivory were significant challenges that also contributed to lower reproduction in 2020.

Despite the fact that 2019 was a mast year for *A. microcymbus*, with individuals producing an average of 893 flowers over the season, relatively few developed into fruit. Fruit set ranged from an average of 0.25 to 0.30 per plant across the three studied sites. Several studies have found low fruit set in rare, endemic *Astragalus* (Searle 2011, Martínez-Sánchez et al. 2011). In *Astragalus nitidiflorus*, an endemic to the Cabezos del Pericón Mountain Range of Murcia Provence in Spain, Martínez-Sánchez et al. (2011), found the ratio of the number of flowers that developed into fruit ranged from 0.173 ± 0.06 to 0.214± 0.09 during two consecutive years. In two endemics to Southwestern Utah, over two consecutive years Searle (2011) found fruit set ranging from an average of 0.11 to 0.23 per plant in *Astragalus holmgreniorum*, and and average of 0.04 to 0.06 per plant in *Astragalus ampullarioides.* Closest to our estimated fruit set, Kaye (1999) found a mean fruit set of 25.8 % ± 2.0 per plant in *Astragalus australis* var. *olympicus*, an endemic to the Olympic Mountains in Washington. *Astragalus microcymbus* had a higher fruit set than observed in *A. nitidiflorus*, *A. holmgreniorum*, and *A. ampullariodes*, although this is still a relatively low number of flowers that become fruit. The resource expenditure required to produce large floral displays during mass flowering may be offset by the benefits from attracting pollinators and satiating seed predators (Pearse et al. 2016).

Seed set was also low in *A. microcymbus*, with an estimated mean seed to ovule ratio of 0.29 across the three studied sites. This ratio is similar to what was observed by Searle (2011) in *A. ampullarioides* seed set which averaged 0.17 and 0.41 over two consecutively studied years (Searle 2011). Higher seed sets have been observed in other rare *Astragalus*. In *A. holmgreniorum,* estimated seed set was 0.38 and 0.66 over two studied years (Searle 2011), and in *A. nitidiflorus*, seed set was 0.610 and 0.788 over two consecutive years (Martínez-Sánchez et al. 2011). In our study, the estimated mean aborted seed to ovule ratio across the three studied sites was 0.33, suggesting that a significant proportion of fertilized ovules are aborted during development. Developing seeds may be aborted if abiotic stress (Sun et al. 2004), self-fertilization or resource availability (Martin and Lee 1993) has caused low fitness in the developing embryo. We also recorded a high ratio of undeveloped ovules, with a mean of 0.37 across the three studied sites. This suggests pollination limitation may be a contributing factor for the low seed set observed in *A. microcymbus*, although this category should be interpreted with caution. During the process of counting, some ovules may have been fertilized but had not yet expanded enough to be distinguishable as aborted ovules, and therefore the number of aborted ovules may be an underestimation, while undeveloped ovules may be an overestimation. Kaye (1999) found a negative relationship between the number of fruits per raceme and the seed set per fruit, suggesting that resource availability may be a limiting factor on reproductive success in *Astragalus australis* var. *olympicus*. Because 2019 was a mast year for *A. microcymbus*, resource availability may have been the cause for the low seed set observed in this study. Our study did not find a correlation between number of fruits per plant and seed set, although there were limitations the 2019 reproductive success data set (discussed below), and further research is needed to more conclusively decipher patterns across fruit and flower to seed and ovule counts. Further research into the mechanisms which caused the observed low reproductive success, including pollinator limitation, abortion, and resource availability are important future steps in understanding the patterns of *A. microcymbus* pre-dispersal reproductive success.

Among the three sites studied in 2019, there was no effect of sites on *A. microcymbus* reproductive success. This is in contrast to work reported by Kaye (1999) who found significant effects of site on reproductive success measures including raceme, flower, fruit and seed production. These differences varied across sites and the interactions between these variables resulted in a different outcome for each site. For example, one site had the highest percent of unfertilized and aborted ovules to total ovules, but still had the highest overall seed set of all sites because they had the lowest amount of seed predation which other sites suffered greatly from. These sites differed greatly in elevation, ranging from 1460 m to 1770 m. Elevation across our studied sites only ranged from 2457 m to 2499 m, which may be a reason for the similarity in reproduction. Due to proximity, DS1 and DS2 may be one interconnected population, therefore this comparison may only be representative of two distinct populations of *A. microcymbus*. Determining difference between sites can aid in determining factors that may threaten its reproduction. This study shows no difference across sites, although a larger study with the inclusion of more sites and years would better elucidate differences if they exist.

In our study, the length of the longest stem was correlated with absolute measures of reproduction including fruit, flower and raceme count, but not correlated with fruit set or seed set. This pattern was also found by Martínez-Sánchez et al. (2011), who found a significant positive correlation between plant size and number of flowers per plant, number of fruit and number of seeds, but no correlation between plant size and fruit set or seed set.

There were limitations to the 2019 reproductive success data that should be considered in the interpretation of these results. Due to the high reproduction that occurred in 2019 and the effort required to collect data, over the season intervals between data collections, and the time that each collection was made across sites varied. To address these irregularities, when comparing across sites I used the maximum flower and maximum fruit counts (the highest counts recorded per plant across all collections), although this method underestimates fruit set for the following reasons. Flower counts included all open flowers, dried flowers and the pedicel scars left behind after a flower had senesced. Fruit counts included all fruit from those newly initiated to those mature and beginning to dehisce, although once a fruit had dehisced it was not clear a fruit had been there in the same way it was a flower. This results in flower counts being more reflective of the true maximum count produced over the whole season, while the maximum fruit count was an underestimation, overall underestimating reproductive success. To address this problem, I also report a second fruit to flower ratio, calculated from counts taken during the period 30 June to 11 July, earlier in the season when less fruit had a chance to mature and fall from the plant. The aim of these two calculations is to provide a range more descriptive of the fruit set of *A. microcymbus* that occurred in 2019. To further address these problems that arose during fruit and flower counts in 2019, in 2020 we changed the methodology used in the reproductive success study from counting all the flowers and fruit on a plant to marking a random sample of racemes and tracking the number of flowers that initiated and developed into mature fruit per raceme. Due to high herbivory and low reproduction in 2020, results were not of sufficient size to analyze.

Small mammal herbivory is a significant threat to *A. microcymbus* populations (U.S. Fish and Wildlife Service 2009). The effects of this threat were observed in studied populations during the growing season of 2020, which greatly impacted vegetative and reproductive growth. Based on the number of diagonal cuts on grazed stems, game camera footage, and frequently observed mountain cottontail rabbits and mountain cottontail rabbit scat in sites, it appears mountain cottontail rabbits were responsible for some or a majority of the herbivory impacts. Mammal herbivory can affect a plants reproduction by reducing the numbers of flowers, fruit and the potential ovules contained within (Fenner and Thompson 2005, Louda 1982). In 2006 and 2007, DBG installed fencing around a subset of sites within South Beaver Creek to exclude herbivores. There was an effect of fencing resulting in an increase in the number of reproductive individuals and an increase in fruit production during mast years, although these effects also coincided with favorable growing conditions, potentially confounding this effect. Fencing also increased snowpack, which may have added more moisture to studied plots, making it difficult to decipher the effects of excluding herbivores (DePrenger-Levin et al. 2013).

Pollination Ecology

Our results suggest that *A. microcymbus* is capable of setting viable seed in the absence of pollinators, however these results are of inadequate sample size to interpret the role autogamy plays in this species effective breeding system. Further, the inconclusive results from the reproductive success study to serve as an open pollinated control limits the ability to interpret the frequency of occurrence between outcrossing and autogamy. Only a few bagged racemes set fruit, and both fruit and seed abortion were observed in those that did, suggesting the capacity for autogamy is limited. This may have also been influenced by unfavorable growing conditions in 2020 and 2021. Further research is needed to describe the breeding system of *A. microcymbus*.

Other studies have found a range of breeding system types across the genus *Astragalus* (Karron 1989, Atasagun et al. 2021, Kaye 1989) and these may be influenced by whether the species is widespread or narrowly distributed (Karron 1989). The ability of a rare population to self-pollinate may be the result of selective pressures on small populations (Karron 1989). Green and Bohart (1975) found that two widespread species, *Astragalus utahensis* and *Astragalus cibarius* were unable to set fruit in a self-pollination experiment, indicating they are self-incompatible. Several studies of rare species of *Astragalus* found they are self-compatible but produced higher fruit set through insect visitation (Kaye 1999, Atasagun et al. 2021, Martínez-Sánchez et al. 2011). In a study of the reproductive success and breeding system of *Astragalus argaeus,* a rare endemic to the Ericiyes Mountains of Turkey, Atasagun et al. (2021) found that it was capable of autogamy, but that fruit set was higher in plants exposed to cross-pollination. Based on the pollen/ovule ratio, this species was also classified as facultatively xenogamous, based on Cruden's scale (Cruden 1977). Martínez-Sánchez et al. (2011) also found higher fruit set in open pollinated *Astragalus nitiflorus* racemes than bagged racemes, but no difference in seed set between the two. In addition to higher reproduction in the presence of insect pollinators, based on Cruden’s Outcrossing Index (Cruden 1977), the flower morphology of *A. nitiflorus* suggests it is facultatively xenogamous (Martínez-Sánchez et al. 2011). Kaye (1999) found that fruit set was significantly lower on bagged racemes of *Astragalus australis* var. *olympicus* than those exposed to insect visitors but found no difference in seed weight or seed set of open pollinated fruit than those produced in the absence insects. Kaye (1999) suggests that the reason that seed set could still be maintained in bagged plants even with reduced fruit set was due to the failed mechanical barriers within the flower that normally would inhibit self-pollination. In *Astragalus linifolius* another narrowly distributed endemic to the Uncompaghre Plateau in Colorado, Karron (1989) found that there was no difference in seed set between self-pollinated or outcrossed fruit and found greater germination success from self-pollinated seeds than outcrossed seeds. However, seedlings of self-pollinated seeds had lower dry weight than seedlings of outcrossed seeds, suggesting the effects of inbreeding depression occur in later life stages. These studies suggest that robust pollinator populations are important in the reproductive success of these *Astragalus* species.

The paucity of results from our study leaves questions regarding the breeding system of *A. microcymbus* unanswered. Further research is needed into this area of this species’ reproductive ecology either during a more favorable growing year, or with the implementation of herbivore exclusion cages. An understanding of the breeding system of a species is important in understanding its pollination ecology and the role pollinators play in successful seed set. The observed pollinator behavior during 2020 suggests that solitary bees are important in the reproduction of *A microcymbus.* If it is determined that *Astragalus microcymbus* relies primarily on outcrossing, or has higher reproductive success through outcrossing, the criticality of conserving these pollinator species is great and conservation efforts for this plant should be extended to support its pollinators as well.

During the studied period, solitary bees were observed directly accessing the reproductive structures of *A. microcymbus* flowers. This was expected based on the corolla morphology of the papilionaceous flowers, which are primarily pollinated by Hymenoptera (Aronne et al. 2012). Other *Astragalus* have been reported to be visited by bumble bees and solitary bees (Kaye 1999). The species observed included *Ashmeadiella sculleni, Ashmeadiella lutzi, Ashmeadiella cactorum* and *Anthidium emarginatum*. *Ashmeadiella* is a genus endemic to North America with high diversity in the Western U.S, especially in the Mojave Basin and Range, the Sonoran Desert and the Central Basin and Range (Murray et al. 2021). Land conversion through solar energy development and urban sprawl is causing habitat loss to these desert dwelling bee species (McCoshum and Geber 2019). *Anthidium emarginatum* is found in lowland areas of the western U.S. including grasslands, Colorado Plateau shrublands, shrub steppe and Chihuahuan Desert (Gonzalez and Griswold 2013). Its recorded distribution spans from Eastern California, Oregon and Washington, through Nevada, Idaho, Utah, Southern Montana, Arizona, New Mexico, Nebraska, to western Kansas and Texas (Gonzalez and Griswold 2013). This species has been observed visiting *Astragalus* and other Fabaceae, as well as other plant families including Asteraceae, Boraginaceae, Brassicaceae, Cactaceae, Lamiaceae, Loasaceae, Malvaceae, Rosaceae, Plantaginaceae, Polemonaceae, and Solanaceae (Gonzalez and Griswold 2013).

In the South Beaver Creek Area of Critical Environmental Concern, habitat degradation from grazing may negatively impact bee populations. In livestock grazed environments, solitary bee populations may be threatened by the reduction in nesting sites and materials, the trampling of existing nests, and the availability of food sources (Black et al. 2011). The availability of nest sites for above or below ground cavity nesters may be a limiting resource for cavity nesting bee populations (Danforth et al. 2019). The genus *Ashmeadiella*, are primarily thought to be soil excavators and below or above ground cavity nesters. They use leaf masticate and mud to build their nests (Danforth et al. 2019). Members of the genus *Anthidium* are above or below ground cavity nesters and are known to use plant and animal fiber in their nest construction, hence their name "wool carder bees" (Danforth et al. 2019).

Two additional bees, *Ceratina nanula* and *Lasioglossum* sp. were observed visiting the flowers without tripping the keel and therefore may not be effective pollinators of *A. microcymbus*. *Ceratina nanula* was recorded as a visitor to *A. microcymbus* in 1989 (Heil and Porter 1989), but based on my observations, this species did not trip the keel and come into contact with the flowers' reproductive structures. These observations were not extensive, and more research is needed to understand the role of *Ceratina nanula* in *A microcymbus* pollination ecology. The *Lasioglossum* observed were not identified to species. These species were observed either inserting their glossa between the wing and banner petals or puncturing a hole in the base of the calyx, possibly feeding from the nectary. The nectary of papilionaceous flowers is located at the base of the corolla (Aronne et al. 2012). These species are much smaller than the *Ashmeadiella* and *Anthidium* observed, so size may play a role in their ability to mechanically elicit the pollination mechanism of this flower.

Post-Primary Dispersal Seed Ecology

Soil Seed Bank Characteristics-

The accumulation of a soil seed bank by a species may buffer populations against genetic and environmental challenges by storing genetic diversity and potential individuals within ungerminated seeds (McCue and Holtsford 1998). Further, the presence of a soil seed bank may play a role in rare *Astragalus* diversification and the maintenance of endemism by maintaining high genetic diversity even within small populations (Jones et al. 2021). This study shows that *A. microcymbus* accumulates some degree of a viable soil seed bank. Seed bank density was highest at the base of the parent plant and decreased with downhill distance from the plant. At 1 meter from the parent plant, very few seeds were found. The scarcity of seeds at the end of this short distance follows a general pattern that desert species tend to have short-distance dispersal (Chambers and MacMahon 1994). Other studies specific to *Astragalus* have also found that seeds are dispersed within close proximity to the parent plant and are subject to the influence of downslope movement by hydrological erosion (Ramos et al. 2010, Searle 2011). In addition to the presence of a soil seed bank, short distance seed dispersal may play a role in demographic factors that contribute to high levels of endemism in the genus by reducing gene flow and keeping seeds in isolated populations (Jones et al. 2021).

It is a general pattern that seed bank density decreases with distance from the parent plant (Bullock and Moy 2004, Caballero et al. 2008) Parent plants deposit seeds in their own vicinity and therefore seed density gradually decreases with increasing distance from the parent plant (Caballero et al. 2008), although due to surface microtopography, microclimate, and community interaction on seed dispersal, this decrease is often more heterogenous than a smooth decline (Bullock and Moy 2004, Caballero et al. 2008). Our study found that at 0.5 meters downhill from the focal plant, seed bank density decreased with increasing bare ground exposure. It is possible that the effect of bare ground was not seen at 0.0 m because this distance is the closest proximity to the seed source, so high seed count was observed at these sites regardless of the microhabitat structure. Conversely, the effect of bare ground may not have been detectable at 1.0 m distance because few seeds where found at this distance regardless of the microhabitat structure.

The absence of seeds in microhabitats with a greater proportion of bare ground exposure suggest that secondary dispersal by overland flow may have occurred to the observed seeds. The connectively of bare ground between vegetation patches in semi-arid environments facilitate the movement of overland flow and sediment while vegetation patches intercept these movements, trapping sediment and acting as sinks to runoff (Bautista et al. 2007). Assuming seeds are subject to the same movements as sediments (Bochet 2015), in the presence of overland flow overtime fewer seeds should occur in microsites with more bare ground exposure, as seeds are carried to and caught in vegetation patches. During the week of 25 July 2020 there were heavy rain events in the Gunnison area and afterwards I observed signs of erosion at the studied sites which suggests that overland flow occurred at these sites the summer prior to soil collections. Wind dispersal may also play a role in seed movement, especially if seeds were still in dry fruit when dispersed from plants which could further facilitate movement by wind (personal observation 2020).

Slope is an important variable in the processes of seed dispersal by overland flow (Bochet 2015) and is characteristic of *A. microcymbus* habitat (U.S. Fish and Wildlife Service 2009). Downslope movement of seeds from overland flow can play a role in dispersal, but it may also lead to seed loss through burial or by the eventual movement out of sites (Bochet 2015). Compared to the estimated 240 to 247 seeds produced per a plant in 2019, the number of seeds found in the seed bank below each plant appeared low. Loss of seeds across slope by secondary dispersal processes may contribute to this low number of seeds, but other factors including senescence, germination and unknown biotic interactions may also be involved in seed fate (Chambers and MacMahon 1994).

At 0.5 meters, more seeds were found at rocks and vegetation combined, although it was unclear from our data which of these non-bare ground structures caused this effect. It is possible that rocks and vegetation both effectively serve as seed catches and that the occurrence of seeds between them is random, although it is possible there are differences in the effect of these structural groups on seed bank density that could be detected with a larger sample size. According to Caballero et al. (2008) in a semi-arid shrub community in Central Spain, above ground vegetation patch structure was a predictor of seed bank composition, with more seeds occurring beneath erect perennial cover at both the center and edges of vegetation patches. Breaking the vegetation category down further into finer groups based on structural growth form could further inform the effect of surrounding vegetation on the spatial distribution of *A. micocymbus* seed bank, although the small sample size of this study limited our ability to do that. Indicating upslope versus downslope position of rocks and vegetation in relationship to sample may also be necessary in detecting an effect of these microhabitat components because seeds are deposited on upslope edges of structural objects (Bochet 2015).

Several characteristics described in this study suggest that the *A. microcymbus* seed bank exhibits some degree of persistence. Persistent seed banks have been observed in other studies of rare *Astragalus* (Searle 2011, Jones et al. 2021). Impermeable seed coats are associated with long-term persistence in seed banks (Martínez-Fernández et al. 2014), and in our study scarification was an important germination requirement. Other studies have also found scarification to be an important requirement for *Astragalus* germination (Atasagun et al. 2021, Kaye 1999, Martínez-Fernández et al. 2014, Seglias personal communication 2020). Based on the low to minimal reproduction that occurred in 2020, it is likely that a portion of these seeds are at least as old as the 2019 cohort, which was a high reproductive year. Seed viability observed in seeds from the soil seed bank (which ranged from 71-89%), was lower than the seed viability observed in seeds collected from parent plants in 2019 (which ranged from 78% to 98%), although these values are still relatively high, suggesting seeds may remain persistent in the soil for a period of time. A long-term study to record temporal fluctuations in seed bank density, as well as further research into the viability of this species' seeds over time and under the environmental fluctuations characteristic of its habitat are important further steps in understanding the temporal component of this species' seed bank.

Microhabitat Characteristics of Established Plants-

Ultimate microhabitat occurrence of an individual is determined not only by seed dispersal patterns but also by germination patterns (Schupp 1995). Our study illustrates that *A. microcymbus* are not randomly distributed across the landscape but occur in microhabitats with a high percent cover of vegetation and in closer proximity to vegetation patches. In the seed bank study, less seeds were found in microsites with higher bare ground exposure, which suggests seed dispersal ecology may play a role in the occurrence patterns of *A. microcymbus,* and vegetation patches or rocks may serve as seed catches. The tendency for established plants to occur in vegetation patches suggests that seeds may be caught in existing vegetation during secondary dispersal, but it also suggests that microsites with higher vegetation may support germination.

In arid environments nurse structures including existing vegetation and rocks alter microsites, making conditions more suitable for seedling establishment and growth (Filazzola et al. 2019, Loayza et al. 2017). In the southern limit of the Atacama Desert in Chile, temperatures beneath rocks and shrubs were lower and have higher water holding capacity than bare interspaces (Loayza et al. 2017). Further, microsites beneath plants had higher organic matter content which can support seed longevity and seedling emergence (Loayza et al. 2017). Vegetation may also influence adult plant survival. *Artemesia tridentata* shrubs can act as a nursery plant for many seedlings and provide protection to plants from grazing (Welch 2009). *Artemesia tridentata* is a foundational species to *A. microcymbus* habitat. There was no tendency detected that *A. microcymbus* grew closer specifically to *A. tridentata* plants when measured in 2020, although *A. tridentata* often made up the vegetation patches that *A. microcymbus* plants were found closer to. The pattern that *A microcymbus* plants occurred closer to vegetation patches and in microsites with higher vegetation cover, may be a result of the general pattern that plant community in arid environments tend to occur in a patchy distribution (Aguiar and Sala 1999) or this characteristic may be specific to the ecology of this species.

No difference was detected in rock cover and proximity to rocks between random microsites and *A. microcymbus* microhabitats suggesting rocks may not influence *A. microcymbus* occurrence, although there could be limits to this interpretation. In this rocky landscape, rocks may occur more widespread and randomly distributed than vegetation, reducing the detectability of a potential preference of *A. microcymbus* for these structures. Further, often rocks that occurred near large vegetation patches, especially those composed of *A. tridentata* and *Yucca harrimaniae*, were buried beneath litter (personal observation 2020). If *A. microcymbus* plants occurred near vegetation, then the presence of litter may reduce the detectability of the nearest rock. Further research with the inclusion of experimental study would be important in determining whether rocks may also adequately serve as nurse structures.

Conclusions

This study described multiple components of *Astragalus microcymbus* reproduction and factors that may challenge its persistence. This plant experiences low reproduction due to low fruit set, low seed set, herbivory, and high year-to-year variation in reproduction. Even during 2019, a mast year, reproductive success as measured by fruit to flower and seed to ovule ratios was fairly low. Small mammal herbivory was severe in 2020, resulting in minimal reproduction.

Due to high herbivory and low reproduction in 2019, we were not able to conduct a successful pollinator exclusion experiment to elucidate the breeding system of *A. microcymbus*. An understanding of the breeding system of a species is important in understanding its reproductive ecology and genetics. Therefore, repeating this experiment in a higher reproduction year, potentially with the exclusion of herbivores, is an important direction for future research. Four species of solitary bees were observed tripping the keel and eliciting the pollination mechanism of the flowers suggesting out-crossing occurs and these bee species may be important in *A. microcymbus* ecology.

A sparse seed bank that decreased with increasing downhill distance from the focal plant was observed. Within 1.0 from the focal plant, few seeds were found suggesting seeds of this species undergoes short distance dispersal. Seed density also was also lower in areas with higher proportion of bare ground exposure. This suggests overland flow may be an agent in secondary dispersal, carrying seeds out of these microsite types where they are caught in microsites with high non-bare ground structure cover (i.e. rocks and vegetation). Established individuals tended to occur in microhabitats closer to vegetation patches and with greater vegetation cover. This suggests that existing vegetation may play an important role in seed dispersal, germination, seedling survival, or adult survival. The scarification requirements of seeds and the minimal reproduction that occurred in 2020, suggest that this seed bank expresses some degree of persistence, although additional research is needed to understand the longevity of *A. microcymbus* seed bank.

Conservation Implications

Astragalus microcymbus reproduction is closely tied to climatic factors, which may be threatened by anthropogenic climate change (DePrenger-Levin and Hufft 2019, U.S. Fish and Wildlife Service 2009). Although this limits options for local management, several management strategies can be recommended based on this study findings that would support *A. microcymbus* reproduction.

Severe impacts from small mammal herbivory were observed in 2020. If these impacts continue, this could threaten the long-term persistence of populations. It has been recorded by ecologists that small mammal populations fluctuate in size cyclically, reaching high densities every three to four years (Krebs and Myers 1974), although I hypothesize that the impacts seen in 2020 were in part indirect effects of drought which caused limited food availability for grazing wildlife. The risk of drought may increase in the future as climate change is expected to bring warmer days and less winter snowpack (U.S. Fish and Wildlife Service 2009). Therefore, the threat of small mammal herbivory may increase for *A. microcymbus*. If there are any viable management options for mediating small mammal populations in *A. microcymbus* habitat, this may support its reproduction.

Details about the breeding system of *A. microcymbus* remains unknown, although based on observed insect visitor behavior, solitary bees play a role in *A. microcymbus* pollination ecology. Maintaining diverse pollinator communities may enhance plant reproductive success

(Albrecht et al. 2012). Low seed set was observed in 2019, and though further research into the mechanisms that caused this is needed, pollination limitation can be limiting factor in plant reproductive success (Fenner and Thompson 2005). Populations of *A. microcymbus* occur on BLM land utilized for livestock grazing. Livestock grazing may reduce solitary bee nesting sites and materials, threaten existing nests, and reduce the availability of food sources (Black et al. 2011). Moderation of grazing intensity is an important management practice to support solitary bee populations (Black et al. 2011, Danforth et al. 2019).

The presence of a soil seed bank in *A. microcymbus* may buffer populations against the demographic challenges of low reproduction years, as was observed in 2020, although continued low reproductive years may deplete these reserves. DePrenger-Levin and Hufft (2019) suggest few mast seedings years may result in a sparse seedbank in *A. microcymbus*. Further, seed bank characteristics may be threatened by climate change, as increased temperatures can reduce seed dormancy and increase germination (Ooi 2012). In this study, community structure may support the post-primary dispersal seed ecology of this species. Non-bare ground structures (rocks or vegetation) may act as seed catches during secondary dispersal, and vegetation specifically may support seedling germination and survival. Preserving plant community structure in *A. microcymbus* habitat may support its post-primary dispersal seed ecology. Plant community degradation from overgrazing; traffic from cattle, recreation and research; and the establishment of invasive annual species, all have the potential to threaten plant community characteristics of this species habitat.

Finally, an understanding of the microhabitat characteristics of *A. microcymbus* plants can inform out-planting efforts if this was needed for genetic reasons. Individuals planted in or near vegetation patches may have a better chance for establishment and survival.

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